

410 Rec'd PCT/PTO 0 2 OCT 2000

FORM-PTO-1390  
(Rev. 10-96)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

**TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371**

003300-685

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5)

09/647544

INTERNATIONAL APPLICATION NO.  
PCT/SE99/00544INTERNATIONAL FILING DATE  
31 March 1999PRIORITY DATE CLAIMED  
2 April 1998 and 28 January 1999

TITLE OF INVENTION  
AN INTEGRIN HETERODIMER AND A SUBUNIT THEREOF

APPLICANT(S) FOR DO/EO/US  
EVY LUNDGREN-ÅKERLUND

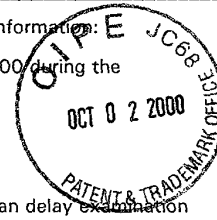
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

It is contemplated that this Application be prosecuted while using Claims 1 to 134 that were presented on May 29, 2000 during the international phase of prosecution as amended in the Preliminary Amendment filed herewith.

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
  2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
  3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).
  4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
  5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
    - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
    - b. ☒ has been transmitted by the International Bureau.
    - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
  6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
  7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
    - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
    - b. ☐ have been transmitted by the International Bureau.
    - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
    - d. ☐ have not been made and will not be made.
  8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
  9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (signed Declaration will follow)
  10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).
- Items 11. to 16. below concern other document(s) or information included:
11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
  12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
  13. ☒ A **FIRST** preliminary amendment.  
☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
  14. ☐ A substitute specification.
  15. ☐ A change of power of attorney and/or address letter.
  16. ☒ Other items or information:

Copies of Swedish Application No. 9801164-6, filed 2 April 1998 and Swedish Application No. 9900319-6, filed 28 January 1999 were submitted during the international phase of prosecution. Thus, the claim for priority has been substantiated.

This Application qualifies for small entity status.



(09/99)

U.S. APPLICATION NO. (if known) <b>09/647544</b>		INTERNATIONAL APPLICATION NO. PCT/SE99/00544		ATTORNEY'S DOCKET NUMBER 003300-685	
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17. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS		PTO USE ONLY	
<b>Basic National Fee (37 CFR 1.492(a)(1)-(5)):</b>  Search Report has been prepared by the EPO or JPO ..... \$860.00 (970)  International preliminary examination fee paid to USPTO (37 CFR 1.482) ..... \$690.00 (956) No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) ..... \$710.00 (958)  Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to U.S. PATENT AND TRADEMARK OFFICE International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) ..... \$100.00 (962)							
<b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>						\$ 1,000.00	
Surcharge of \$130.00 (154) for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492(e)).           20 <input type="checkbox"/> 30 <input type="checkbox"/>				\$ --			
Claims	Number Filed	Number Extra	Rate				
Total Claims	134 - 20 =	114	X\$18.00 (966)	\$ 2,052.00			
Independent Claims	38 - 3 =	35	X\$80.00 (964)	\$ 2,800.00			
Multiple dependent claim(s) (if applicable)				+ \$270.00 (968)		\$ --	
<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$ 5,852.00			
Reduction for 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				\$ 2,926.00			
<b>SUBTOTAL =</b>				\$ 2,926.00			
Processing fee of \$130.00 (156) for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492(f)).           20 <input type="checkbox"/> 30 <input type="checkbox"/>				\$ --			
<b>TOTAL NATIONAL FEE =</b>				\$ 2,926.00			
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 (581) per property +				\$ --			
<b>TOTAL FEES ENCLOSED =</b>				\$ 2,926.00			
				Amount to be: refunded		\$	
				charged		\$	

a. ☒ A check in the amount of \$ 2,926.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 02-4800 in the amount of \$ \_\_\_\_\_ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

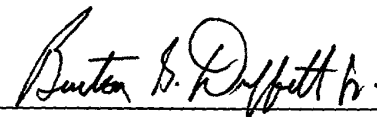
  

SEND ALL CORRESPONDENCE TO:

Benton S. Duffett, Jr.  
 BURNS, DOANE, SWECKER & MATHIS, L.L.P.  
 P.O. Box 1404  
 Alexandria, Virginia 22313-1404

Filed: October 2, 2000

  
 SIGNATURE  
 Benton S. Duffett, Jr.  
 NAME  
22,030  
 REGISTRATION NUMBER

FORM-PTO-1390  
(Rev. 10-96)

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523 Rec'd PCT/PTO 02 OCT 2000

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09/647544

INTERNATIONAL APPLICATION NO.

PCT/SE99/00544

ATTORNEY'S DOCKET NUMBER

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Alexandria, Virginia 22313-1404

SIGNATURE

Benton S. Duffett, Jr.

NAME

Filed: October 2, 2000

22,030

REGISTRATION NUMBER

(09/99)

09/647544

528 Rec'd PCT/PTO 02 OCT 2000

Patent  
Attorney's Docket No. 003300-685

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of	)	BOX PCT
	)	Attention: DO/EO/US
EVY LUNDGREN-ÅKERLUND	)	
	)	
Application No.: Unassigned	)	Group Art Unit: Unassigned
	)	
Filed: October 2, 2000	)	Examiner: Unassigned
	)	
For: AN INTEGRIN HETERODIMER	)	
AND A SUBUNIT THEREOF	)	
	)	
	)	

**PRELIMINARY AMENDMENT**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

This application corresponds to International Application No. PCT/SE99/00544,  
filed March 31, 1999.

It is contemplated that this Application be prosecuted while using Claims 1 to 134  
that were submitted on May 29, 2000 during the international phase of prosecution as  
further amended herein.

**In the Abstract:**

Please add the Abstract of the Disclosure that is provided on a separate sheet.

In the Claims:

Claim 8, lines 1 and 2, delete "in any one of claims 6 and 7" and insert --claim 6--.

Claim 20, line 1, delete "or 19".

Claim 21, line 1, delete "or 19".

Claim 26, line 2, delete "any one of claims 22-25" and insert --claim 22--.

Claim 27, lines 2 and 3, delete "any one of claims 22-25" and insert --claim 22--.

Claim 28, line 3, delete "any one of claims 22-25" and insert --claim 22--.

Claim 38, lines 1 and 2, delete "any one of claims 31-37" and insert --claim 31--.

Claim 41, lines 1 and 2, delete "any one of claims 31-37" and insert --claim 31--.

Claim 42, lines 1 and 2, delete "any one of claims 31-37" and insert --claim 31--.

Claim 43, lines 1 and 2, delete "any one of claims 31-37" and insert --claim 31--.

Claim 44, lines 1 and 2, delete "any one of claims 31-37" and insert --claim 31--.

Claim 45, line 1, delete "any one of claims 31-37 and insert --claim 31--.

Claim 52, lines 1 and 2, delete "any one of claims 46-51" and insert --claim 46--.

Claim 53, lines 1 and 2, delete "any one of claims 46-51" and insert --claim 46--.

Claim 60, lines 1 and 2, delete "any one of claims 54-59" and insert --claim 54--.

Claim 72, lines 1 and 2, delete "any one of claims 64-71" and insert --claim 64--.

Claim 93, line 1, delete "any one of claims 86-92" and insert --claim 86--.

Claim 96, line 1, delete "any one of claims 86-92" and insert --claim 86--.

Claim 97, line 1, delete "any one of claims 86-92" and insert --claim 86--.

Claim 98, line 1, delete "any one of claims 86-92" and insert --claim 86--.

Claim 105, lines 1 and 2, delete "any one of claims 99-104" and insert --claim  
99--.

Claim 106, lines 1 and 2, delete "any one of claims 99-104" and insert --claim  
99--.

Claim 113, lines 1 and 2, delete "any one of claims 107-112" and insert --claim  
107--.

Claim 125, lines 1 and 2, delete "any one of claims 117-124" and insert --claim  
117--.

**REMARKS**

The present Amendment adds an Abstract of the Disclosure on a separate sheet and  
eliminates the use of multiple dependency.

The examination and allowance of the application are respectfully requested.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: Benton S. Duffett Jr.  
Benton S. Duffett, Jr.  
Registration No. 22,030

P.O. Box 1404  
Alexandria, Virginia 22313-1404  
(703) 836-6620

Date: October 2, 2000



### Abstract of the Disclosure

A recombinant or isolated integrin heterodimer comprising a novel subunit  $\alpha 10$  in association with a subunit  $\beta$  is described. The  $\alpha 10$  integrin may be purified from bovine chondrocytes on a collagen-type-II affinity column. The integrin or the subunit  $\alpha 10$  can be used as marker or target of all types of cells, e.g. of chondrocytes, osteoblasts and fibroblasts. The integrin or subunit  $\alpha 10$  thereof can be used as marker or target in different physiological or therapeutic methods. They can also be used as active ingredients in pharmaceutical compositions and vaccines.

11/11/2019 11:11:11 AM

Attorney's Docket No. 003300-685

Applicant or Patentee: EVY LUNDGREN-ÅKERLUND

Application or Patent No.: \_\_\_\_\_

Filed or Issued: October 26, 2000

For: AN INTEGRIN HETERODIMER AND A SUBUNIT THEREOF

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS  
 (37 C.F.R. §§ 1.9(f) AND 1.27(c)) - SMALL BUSINESS CONCERN**

I hereby declare that I am

- ☒ the owner of the small business concern identified below:  
☐ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN CARTELA AB

ADDRESS OF CONCERN c/o Evy Lundgren-Åkerlund

Trollsjövägen 165, 237 33 Bjärred, Sweden

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 C.F.R. § 121.12, and reproduced in 37 C.F.R. § 1.9(d), for purposes of paying reduced fees under Sections 41(a) and 41(b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average, over the previous fiscal year of the concern, of the persons employed on a full-time, part-time, or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled An integrin heterodimer and a subunit thereof

by inventor(s) Evy Lundgren-Åkerlund  
 described in

- ☐ the specification filed herewith  
☒ Application No. PCT/SE99/00544, filed 31 March 1999  
☐ Patent No. \_\_\_\_\_, issued \_\_\_\_\_.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern, or organization having rights to the invention is listed below,\* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 C.F.R. § 1.9(c), or by any concern that would not qualify as either a small business concern under 37 C.F.R. § 1.9(d) or a nonprofit organization under 37 C.F.R. § 1.9(e).

\*NOTE: Separate verified statements are required from each named person, concern, or organization having rights to the invention averring to their status as small entities. (37 C.F.R. § 1.27.)

Application Serial No. \_\_\_\_\_  
Attorney's Docket No. 003300-685

NAME \_\_\_\_\_

ADDRESS \_\_\_\_\_

☐ individual      ☐ small business concern      ☐ nonprofit organization

NAME \_\_\_\_\_

ADDRESS \_\_\_\_\_

☐ individual      ☐ small business concern      ☐ nonprofit organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earlier of the issue fee and any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b).)

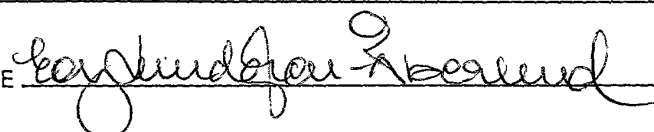
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING EVY LUNDGREN - ÅKERLUND

TITLE OF PERSON OTHER THAN OWNER MANAGING DIRECTOR

ADDRESS OF PERSON SIGNING Trollsjövägen 165

237 33 BJÄRRED, SWEDEN

SIGNATURE  DATE 2000-10-09

AN INTEGRIN HETERODIMER AND A SUBUNIT THEREOF

## FIELD OF THE INVENTION

The present invention relates to a recombinant or isolated integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , the subunit  $\alpha 10$  thereof, homologues and fragments of said integrin and of said subunit  $\alpha 10$  having similar biological activity, processes of producing the same, polynucleotides and oligonucleotides encoding the same, vectors and cells comprising the same, binding entities binding specifically to the same, and the use of the same.

## BACKGROUND OF THE INVENTION

The integrins are a large family of transmembrane glycoproteins that mediate cell-cell and cell-matrix interactions (1-5). All known members of this superfamily are non-covalently associated heterodimers composed of an  $\alpha$ - and a  $\beta$ -subunit. At present, 8  $\beta$ -subunits ( $\beta 1$ - $\beta 8$ ) (6) and 16  $\alpha$ -subunits ( $\alpha 1$ - $\alpha 9$ ,  $\alpha v$ ,  $\alpha M$ ,  $\alpha L$ ,  $\alpha X$ ,  $\alpha IIb$ ,  $\alpha E$  and  $\alpha D$ ) have been characterized (6-21), and these subunits associate to generate more than 20 different integrins. The  $\beta 1$ -subunit has been shown to associate with ten different  $\alpha$ -subunits,  $\alpha 1$ - $\alpha 9$  and  $\alpha v$ , and to mediate interactions with extracellular matrix proteins such as collagens, laminins and fibronectin. The major collagen binding integrins are  $\alpha 1 \beta 1$  and  $\alpha 2 \beta 1$  (22-25). The integrins  $\alpha 3 \beta 1$  and  $\alpha 9 \beta 1$  have also been reported to interact with collagen (26,27) although this interaction is not well understood (28). The extracellular N-terminal regions of the  $\alpha$  and  $\beta$  integrin subunits are important in the binding of ligands (29,30). The N-terminal region of the  $\alpha$ -subunits is composed of a seven-fold repeated sequence (12,31) containing FG and GAP consensus sequences. The repeats are predicted to fold into a  $\beta$ -propeller domain

(32) with the last three or four repeats containing putative divalent cation binding sites. The  $\alpha$ -integrin subunits  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha D$ ,  $\alpha E$ ,  $\alpha L$ ,  $\alpha M$  and  $\alpha X$  contain a ~200 amino acid inserted domain, the I-domain (A-domain), which  
5 shows similarity to sequences in von Willebrand factor, cartilage matrix protein and complement factors C2 and B (33,34). The I-domain is localized between the second and third FG-GAP repeats, it contains a metal ion-dependent adhesion site (MIDAS) and it is involved in binding of  
10 ligands (35-38).

Chondrocytes, the only type of cells in cartilage, express a number of different integrins including  $\alpha 1\beta 1$ ,  $\alpha 2\beta 1$ ,  $\alpha 3\beta 1$ ,  $\alpha 5\beta 1$ ,  $\alpha 6\beta 1$ ,  $\alpha v\beta 3$ , and  $\alpha v\beta 5$  (39-41). It has been shown that  $\alpha 1\beta 1$  and  $\alpha 2\beta 1$  mediate chondrocyte inter-  
15 actions with collagen type II (25) which is one of the major components in cartilage. It has also been shown that  $\alpha 2\beta 1$  is a receptor for the cartilage matrix protein chondroadherin (42).

## 20 SUMMARY OF THE INVENTION

The present invention relates to a novel collagen type II binding integrin, comprising a subunit  $\alpha 10$  in association with a subunit  $\beta$ , especially subunit  $\beta 1$ , but also other  $\beta$ -subunits may be contemplated. In preferred  
25 embodiments, this integrin has been isolated from human or bovine articular chondrocytes, and human chondrosarcoma cells.

The invention also encompasses integrin homologues of said integrin, isolated from other species, such as  
30 bovine integrin heterodimer comprising a subunit  $\alpha 10$  in association with a subunit  $\beta$ , preferably  $\beta 1$ , as well as homologues isolated from other types of human cells or from cells originating from other species.

The present invention relates in particular to a  
35 recombinant or isolated integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, and homologues and or fragments thereof having the

same biological activity.

The invention further relates to a process of producing a recombinant integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID  
5 No. 2, or homologues or fragments thereof having similar biological activity, which process comprises the steps of

a) isolating a polynucleotide comprising a nucleotide sequence coding for a integrin subunit  $\alpha 10$ , or homologues or fragments thereof having similar biological  
10 activity,

b) constructing an expression vector comprising the isolated polynucleotide,

c) transforming a host cell with said expression vector,

d) culturing said transformed host cell in a culture medium under conditions suitable for expression of integrin subunit  $\alpha 10$ , or homologues or fragments thereof having similar biological activity, in said transformed host cell, and, optionally,  
15

e) isolating the integrin subunit  $\alpha 10$ , or homologues or fragments thereof having the same biological activity, from said transformed host cell or said culture medium.  
20

The integrin subunit  $\alpha 10$ , or homologues or fragments thereof having the same biological activity, can also be provided by isolation from a cell in which they are naturally present.  
25

The invention also relates to an isolated polynucleotide comprising a nucleotide coding for a integrin subunit  $\alpha 10$ , or homologues or fragments thereof having similar biological activity, which polynucleotide comprises the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or parts thereof.  
30

The invention further relates to an isolated polynucleotide or oligonucleotide which hybridises to a DNA or RNA encoding an integrin subunit  $\alpha 10$ , having the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or homologues or fragments thereof, wherein said polyoligo-  
35

nucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding the integrin subunit  $\alpha 1$ .

The invention relates in a further aspect to vectors comprising the above polynucleotides, and to cells containing said vectors and cells that have polynucleotides or oligonucleotides as shown in SEQ ID No. 1 or 2 integrated in their genome.

The invention also relates to binding entities having the capability of binding specifically to the integrin subunit  $\alpha 10$  or to homologues or fragments thereof, such as proteins, peptides, carbohydrates, lipids, natural ligands, polyclonal antibodies or monoclonal antibodies.

In a further aspect, the invention relates to a recombinant or isolated integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , in which the subunit  $\alpha 10$  comprises the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or homologues or fragments thereof having similar biological activity.

In a preferred embodiment thereof, the subunit  $\beta$  is  $\beta 1$ .

The invention also relates to a process of producing a recombinant integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , in which the subunit  $\alpha 10$  comprises the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, which process comprises the steps of

a) isolating one polynucleotide comprising a nucleotide sequence coding for a subunit  $\alpha 10$  of an integrin heterodimer and, optionally, another polynucleotide comprising a nucleotide sequence coding for a subunit  $\beta$  of an integrin heterodimer, or for homologues or fragments thereof having similar biological activity,

b) constructing an expression vector comprising said isolated polynucleotide coding for said subunit  $\alpha 10$  in combination with an expression vector comprising said isolated nucleotide coding for said subunit  $\beta$ ,

c) transforming a host cell with said expression vectors,

d) culturing said transformed host cell in a culture medium under conditions suitable for expression of an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or homologues or fragments thereof similar biological activity, in said transformed host cell, and, optionally,

e) isolating the integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or homologues or fragments thereof having the same biological activity, from said transformed host cell or said culture medium.

The integrin heterodimer, or homologues or fragments thereof having similar biological activity, can also be provided by isolation from a cell in which they are naturally present.

The invention further relates to a cell containing a first vector, said first vector comprising a polynucleotide coding for a subunit  $\alpha 10$  of an integrin heterodimer, or for homologues or parts thereof having similar biological activity, which polynucleotide comprises the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2 or parts thereof, and, optionally, a second vector, said second vector comprising a polynucleotide coding for a subunit  $\beta$  of an integrin heterodimer, or for homologues or fragments thereof.

In still another aspect, the invention relates to binding entities having the capability of binding specifically to the integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having similar biological activity, preferably wherein the subunit  $\beta$  is  $\beta 1$ . Preferred binding entities are proteins, peptides, carbohydrates, lipids, natural ligands, polyclonal antibodies and monoclonal antibodies.

In a further aspect, the invention relates to a fragment of the integrin subunit  $\alpha 10$ , which fragment is a peptide chosen from the group comprising peptides of



the cytoplasmic domain, the I-domain and the spliced domain.

In one embodiment, said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

5 In another embodiment, said fragment comprises the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

In a further embodiment, said fragment comprises the amino acid sequence from about amino acid No. 140  
10 to about amino acid No. 337 in SEQ ID No. 1.

Another embodiment of the invention relates to a polynucleotide or oligonucleotide coding for a fragment of the human integrin subunit  $\alpha 10$ . In one embodiment this polynucleotide or oligonucleotide codes for a fragment  
15 which is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain. In further embodiments the polynucleotide or oligonucleotide codes for the fragments defined above.

The invention also relates to binding entities having the capability of binding specifically to a fragment  
20 of the integrin subunit  $\alpha 10$  as defined above.

The invention also relates to a process of using an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin  
25 heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or a homologue or fragment of said integrin or subunit having similar biological activity, as a marker or target molecule of cells or tissues expressing said integrin subunit  $\alpha 10$ , which cells or tissues are of animal includ-  
30 ing human origin.

In an embodiment of this process the fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

In further embodiments of said process the frag-  
35 ment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ, or a fragment comprising the amino acid sequence from about amino acid No. 952 to

about amino acid No. 986 of SEQ ID No. 1, or a fragment comprising the amino acid sequence from about amino acid no. 140 to about amino acid no. 337 of SEQ ID no. 1.

The subunit  $\beta$  is preferably  $\beta 1$ . The cells are preferably chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

Said process may be used during pathological conditions involving said subunit  $\alpha 10$ , such as pathological conditions comprising damage of cartilage, or comprising trauma, rheumatoid arthritis and osteoarthritis.

Said process may be used for detecting the formation of cartilage during embryonal development, or for detecting physiological or therapeutic reparation of cartilage.

Said process may also be used for selection and analysis, or for sorting, isolating or purification of chondrocytes.

A further embodiment of said process is a process for detecting regeneration of cartilage or chondrocytes during transplantation of cartilage or chondrocytes.

A still further embodiment of said process is a process for in vitro studies of differentiation of chondrocytes.

The invention also comprises a process of using binding entities having the capability of binding specifically to an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having similar biological activity, as markers or target molecules of cells or tissues expressing said integrin subunit  $\alpha 10$ , which cells or tissues are of animal including human origin.

The fragment in said process may be a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain. In preferred embodiments said fragment is a peptide comprising the

amino acid sequence KLGFFAHKKIPEEEKREEKLEQ, or a fragment comprising the amino acid sequence from about amino acid No. 952 to about amino acid No. 986 of SEQ ID No. 1, or a fragment comprising the amino acid sequence from about amino acid No. 140 to about amino acid no. 337 of SEQ ID No. 1.

The process may also be used for detecting the presence of an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or of an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or of homologues or fragments thereof having similar biological activity.

In a further embodiment said process is a process for determining the differentiation-state of cells during embryonic development, angiogenesis, or development of cancer.

In a still further embodiment this process is a process for detecting the presence of an integrin subunit  $\alpha 10$ , or of a homologue or fragment of said integrin subunit having similar biological activity, on cells, whereby a polynucleotide or oligonucleotide chosen from the group comprising a polynucleotide or oligonucleotide chosen from the nucleotide sequence shown in SEQ ID No. 1 is used as a marker under hybridisation conditions wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit  $\alpha 1$ . Said cells may be chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts. Said integrin fragment may be a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain, such as a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ, or a fragment comprising the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1, or a fragment comprising the amino acid sequence from about amino acid No. 140 to about amino acid no. 337 of SEQ ID No. 1.

In a still further embodiment the process is a process for determining the differentiation-state of cells during development, in pathological conditions, in tissue regeneration or in therapeutic and physiological reparation of cartilage. The pathological conditions may be any pathological conditions involving the integrin subunit  $\alpha 10$ , such as rheumatoid arthritis, osteoarthritis or cancer. The cells may be chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

The invention also relates to a process for determining the differentiation-state of cells during development, in pathological conditions, in tissue regeneration and in therapeutic and physiological reparation of cartilage, whereby a polynucleotide or oligonucleotide chosen from the nucleotide sequence shown in SEQ ID No. 1 is used as a marker under hybridisation conditions wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit  $\alpha 1$ . Embodiments of this aspect comprise a process, whereby said polynucleotide or oligonucleotide is a polynucleotide or oligonucleotide coding for a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain, such as a polynucleotide or oligonucleotide coding for a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ, or comprising the amino acid sequence from about amino acid No. 952 to about amino acid no. 986 of SEQ ID No. 1, or the amino acid sequence from about amino acid No. 140 to about amino acid No. 337 of SEQ ID No. 1. Said pathological conditions may be any pathological conditions involving the integrin subunit  $\alpha 10$ , such as rheumatoid arthritis, osteoarthritis or cancer, or atherosclerosis or inflammation. Said cells may be chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

In a further aspect the invention relates to a pharmaceutical composition comprising as an active ingredient a pharmaceutical agent or an antibody which is capable of using an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit  $\alpha 10$  having similar biological activity, as a target molecule. An embodiment of said pharmaceutical composition is intended for use in stimulating, inhibiting or blocking the formation of cartilage, bone or blood vessels. A further embodiment comprises a pharmaceutical composition for use in preventing adhesion between tendon/ligaments and the surrounding tissue after infection, inflammation and after surgical intervention where adhesion impairs the function of the tissue.

The invention is also related to a vaccine comprising as an active ingredient an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit  $\alpha 10$ , or DNA or RNA coding for said integrin subunit  $\alpha 10$ .

A further aspect of the invention is related to the use of the integrin subunit  $\alpha 10$  as defined above as a marker or target in transplantation of cartilage or chondrocytes.

A still further aspect of the invention is related to a method of using binding entities having the capability of binding specifically to an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having similar biological activity, for promoting adhesion of chondrocytes and/or osteoblasts to surfaces of implants to stimulate osseointegration.

The invention is also related to the use of an integrin subunit  $\alpha 10$  or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$  as a target for anti-

adhesive drugs or molecules in tendon, ligament, skeletal muscle or other tissues where adhesion impairs the function of the tissue.

The invention also relates to a method of stimulating, inhibiting or blocking the formation of cartilage or bone, comprising administration to a subject a suitable amount of a pharmaceutical agent or an antibody which is capable of using an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit  $\alpha 10$  having similar biological activity, as a target molecule.

In another embodiment the invention is related to a method of preventing adhesion between tendon/ligaments and the surrounding tissue after infection, inflammation and after surgical intervention where adhesion impairs the function of the tissue, comprising administration to a subject a suitable amount of a pharmaceutical agent or an antibody which is capable of using a integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit  $\alpha 10$  having similar biological activity, as a target molecule.

The invention also relates to a method of stimulating extracellular matrix synthesis and repair by activation or blockage of an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or of the subunit  $\alpha 10$  thereof, or of a homologue or fragment of said integrin or subunit  $\alpha 10$  having similar biological activity.

In a further aspect the invention relates to a method of in vitro detecting the presence of integrin binding entities, comprising interaction of an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit, with a sample, thereby causing said integrin, subunit  $\alpha 10$ , or homologue or fragment thereof having similar biological activity, to modulate

the binding to its natural ligand or other integrin binding proteins present in said sample.

The invention also relates to a method of in vitro studying consequences of the interaction of a human  
5 heterodimer integrin comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit, with an integrin binding entity and thereby initiate a cellular reaction. Said consequences may be measured as alterations in cellular functions.  
10

A still further aspect of the inventions relates to a method of using DNA or RNA encoding an integrin subunit  $\alpha 10$  or homologues or fragments thereof as a molecular target. In an embodiment of this aspect, a polynucleotide  
15 or oligonucleotide hybridises to the DNA or RNA encoding an integrin subunit  $\alpha 10$  or homologues or fragments thereof, whereby said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit  $\alpha 1$ .

20 The invention also relates to a method of using a human heterodimer integrin comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit, or a DNA or RNA encoding an integrin subunit  $\alpha 10$  or homologues or fragments thereof, as a marker or target molecule during  
25 angiogenesis.

#### BRIEF DESCRIPTION OF THE FIGURES

Fig.1 Affinity purification of the  $\alpha 10$  integrin subunit on collagen type II-Sepharose.

30 Fig. 2. Amino acid sequences of peptides from the bovine  $\alpha 10$  integrin subunit.

Fig. 3a. Affinitypurification and immunoprecipitation of the integrin subunit  $\alpha 10$  from bovine chondrocytes.

35 Fig. 3b. Affinitypurification and immunoprecipitation of the integrin subunit  $\alpha 10$  from human chondrocytes.

Fig. 3c. Affinitypurification and immunoprecipitation of the integrin subunit  $\alpha 10$  from human chondrosarcoma cells.

Fig. 4. A 900 bp PCR-fragment corresponding to the  
5 bovine integrin subunit  $\alpha 10$

Fig. 5. Schematic map of the three overlapping  $\alpha 10$  clones.

Fig. 6. Nucleotide sequence and deduced amino acid sequence of the human  $\alpha 10$  integrin subunit.

10 Fig. 7. Northern blot of integrin  $\alpha 10$  mRNA.

Fig. 8 Immunoprecipitation of the  $\alpha 10$  integrin subunit from human chondrocytes using antibodies against the cytoplasmic domain of  $\alpha 10$  (a). Western blot of the  $\alpha 10$  associated  $\beta$ -chain (b).

15 Fig. 9. Immunostaining of  $\alpha 10$  integrin in human articular cartilage.

Fig. 10 Immunostaining of  $\alpha 10$  integrin in 3 day mouse limb cartilage.

20 Fig 11. Immunostaining of  $\alpha 10$  integrin in 13.5 day mouse embryo.

Fig 12. Hybridisation of  $\alpha 10$  mRNA in various human tissues.

25 Fig. 13 Immunostaining of fascia around tendon (a), skeletal muscle (b) and heart valves (c) in 3 day mouse limb.

Fig. 14. PCR fragments corresponding to  $\alpha 10$  integrin subunit from human chondrocytes, human endothelial cells, human fibroblasts and rat tendon.

30 Fig 15. Partial genomic nucleotide sequence of the human integrin subunit  $\alpha 10$ .

Fig 16. Upregulation of  $\alpha 10$  integrin subunit in chondrocytes cultured in alginate.

35 Fig 17. Immunoprecipitation of the  $\alpha 10$  integrin subunit from human smooth muscle cells

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention demonstrate that human and



bovine chondrocytes express a novel, collagen type II-binding integrin in the  $\beta 1$ -family. An earlier study presented some evidence for that human chondrosarcoma cells also express this integrin (25). Immunoprecipitation experiments using antibodies against the integrin subunit  $\beta 1$  revealed that this novel  $\alpha$ -integrin subunit had an apparent molecular weight ( $M_r$ ) of approximately 160 kDa under reducing conditions, and was slightly larger than the  $\alpha 2$  integrin subunit. To isolate this  $\alpha$ -subunit collagen type II-binding proteins were affinity purified from bovine chondrocytes. The chondrocyte lysate was first applied to a fibronectin-Sepharose precolumn and the flow through was then applied to a collagen type II-Sepharose column. A protein with  $M_r$  of approximately 160 kD was specifically eluted with EDTA from the collagen column but not from the fibronectin column. The  $M_r$  of this protein corresponded with the  $M_r$  of the unidentified  $\beta 1$ -related integrin subunit. The 160 kD protein band was excised from the SDS-PAGE gel, digested with trypsin and the amino acid sequences of the isolated peptides were analysed.

Primers corresponding to isolated peptides amplified a 900 bp PCR-fragment from bovine cDNA which was cloned, sequenced and used for screening of a human articular chondrocyte  $\lambda$ ZapII cDNA library to obtain the human integrin  $\alpha$ -subunit homologue. Two overlapping clones, hc1 and hc2 were isolated, subcloned and sequenced. These clones contained 2/3 of the nucleotide sequence including the 3' end of the cDNA. A third clone which contained the 5' end of the  $\alpha 10$  cDNA, was obtained using the RACE technique. Sequence analysis of the 160 kD protein sequence showed that it was a member of the integrin  $\alpha$ -subunit family and the protein was named  $\alpha 10$ .

The deduced amino acid sequence of  $\alpha 10$  was found to share the general structure of the integrin  $\alpha$ -subunits described in previously published reports (6-21). The large extracellular N-terminal part of  $\alpha 10$  contains a

seven-fold repeated sequence which was recently predicted to fold into a  $\beta$ -propeller domain (32). The integrin subunit  $\alpha 10$  contains three putative divalent cation-binding sites (DxD/NxD/NxxxD) (53), a single spanning transmembrane domain and a short cytoplasmic domain. In contrast to most  $\alpha$ -integrin subunits the cytoplasmic domain of  $\alpha 10$  does not contain the conserved sequence KxGFF (R/K) R. The predicted amino acid sequence in  $\alpha 10$  is KLGFFAH. Several reports indicate that the integrin cytoplasmic domains are crucial in signal transduction (54) and that membrane-proximal regions of both  $\alpha$ - and  $\beta$ -integrin cytoplasmic domains are involved in modulating conformation and affinity state of integrins (55-57). It is suggested that the GFFKR motif in  $\alpha$ -chains are important for association of integrin subunits and for transport of the integrin to the plasma membrane (58). The KxGFFKR domain has been shown to interact with the intracellular protein calreticulin (59) and interestingly, calreticulin-null embryonic stem cells are deficient in integrin-mediated cell adhesion (60). It is therefor possible that the sequence KLGFFAH in  $\alpha 10$  have a key function in regulating the affinity between  $\alpha 10\beta 1$  and matrix proteins.

Integrin  $\alpha$  subunits are known to share an overall identity of 20-40% (61). Sequence analysis showed that the  $\alpha 10$  subunit is most closely related to the I-domain containing  $\alpha$ -subunits with the highest identity to  $\alpha 1$  (37%) and  $\alpha 2$  (35%). The integrins  $\alpha 1\beta 1$  and  $\alpha 2\beta 1$  are known receptors for both collagens and laminins (24;62;63) and we have also recently demonstrated that  $\alpha 2\beta 1$  interacts with the cartilage matrix protein chondroadherin (42). Since  $\alpha 10\beta 1$  was isolated on a collagen type II-Sepharose, we know that collagen type II is a ligand for  $\alpha 10\beta 1$ . We have also shown by affinity purification experiments that  $\alpha 10\beta 1$  interacts with collagen type I but it remains to be seen whether laminin or chondroadherin are also ligands for this integrin.

The  $\alpha 10$  associated  $\beta$ -chain migrated as the  $\beta 1$  integrin subunit both under reducing and non-reducing conditions. To verify that the  $\alpha 10$  associated  $\beta$ -chain indeed is  $\beta 1$ , chondrocyte lysates were immunoprecipitated with  
5 antibodies against  $\alpha 10$  or  $\beta 1$  followed by Western blot using antibodies against the  $\beta 1$ -subunit. These results clearly demonstrated that  $\alpha 10$  is a member of the  $\beta 1$ -integrin family. However, the possibility that  $\alpha 10$  combine also with other  $\beta$ -chains can not be excluded..

10 A polyclonal peptide antibody raised against the cytoplasmic domain of  $\alpha 10$  precipitated two protein bands with  $M_r$  of approximately 160 kD ( $\alpha 10$ ) and 125 kD ( $\beta 1$ ) under reducing conditions. Immunohistochemistry using the  $\alpha 10$ -antibody showed staining of the chondrocytes in tissue sections of human articular cartilage. The antibody  
15 staining was clearly specific since preincubation of the antibody with the  $\alpha 10$ -peptide completely abolished the staining. Immunohistochemical staining of mouse limb sections from embryonic tissue demonstrated that  $\alpha 10$  is upregulated during condensation of the mesenchyme. This  
20 indicate that the integrin subunit  $\alpha 10$  is important during the formation of cartilage. In 3 day old mice  $\alpha 10$  was found to be the dominating collagen binding integrin subunit which point to that  $\alpha 10$  has a key function in  
25 maintaining normal cartilage functions.

Expression studies on the protein and mRNA level show that the distribution of  $\alpha 10$  is rather restrictive. Immunohistochemistry analyses have shown that  $\alpha 10$  integrin subunit is mainly expressed in cartilage but it is  
30 also found in perichondrium, periosteum, ossification groove of Ranvier, in fascia surrounding tendon and skeletal muscle and in the tendon-like structures in the heart valves. This distribution point to that  $\alpha 10$  integrin subunit is present also on fibroblasts and  
35 osteoblasts. PCR amplification of cDNA from different cell types revealed the presence of an alternatively spliced  $\alpha 10$  integrin subunit. This spliced  $\alpha 10$  was domi-

nating in fibroblasts which suggests that  $\alpha 10$  in fibroblasts may have a different function compared to  $\alpha 10$  present on chondrocytes.

Expression of the integrin subunit  $\alpha 10$  was found to  
5 decrease when chondrocytes were cultured in monolayer. In contrast, the expression of  $\alpha 10$  was found to increase when the cells were cultured in alginate beads. Since the latter culturing model is known to preserve the phenotype of chondrocytes the results suggest that  $\alpha 10$  can function  
10 as marker for a differentiated chondrocyte.

Adhesion between tendon/ligaments and the surrounding tissue is a well-known problem after infection, injury and after surgical intervention. Adhesion between tendon and tendon sheets impairs the gliding function and  
15 cause considerable problems especially during healing of tendons in e.g. the hand and fingers leading to functional incapacity. The localisation of the  $\alpha 10$  integrin subunit in the fascia of tendon and skeletal muscle makes  $\alpha 10$  a possible target for drugs and molecules with anti-  
20 adhesive properties that could prevent impairment of the function of tendon/ligament. The integrin subunit  $\alpha 10$  can also be a target for drugs or molecules with anti-adhesive properties in other tissues where adhesion is a  
25 problem.

## EXAMPLES

### Example 1

Affinity purification of the  $\alpha 10$  integrin subunit on  
30 collagen type II-Sepharose.

#### Materials and Methods

Bovine chondrocytes, human chondrocytes or human chondrosarcoma cells were isolated as described earlier [Holmvall et al, Exp Cell Res, 221, 496-503 (1995),  
35 Camper et al, JBC, 273, 20383-20389 (1998)]. A Triton X-100 lysate of bovine chondrocytes was applied to a fibronectin-Sepharose precolumn followed by a collagen

type II-Sepharose column and the integrin subunit  $\alpha 10$  was eluted from the collagen type II-column by EDTA (Camper et al, JBC, 273, 20383-20389 (1998). The eluted proteins were precipitated by methanol/chloroform, separated by SDS-PAGE under reducing conditions and stained with Coomassie blue. (Camper et al, JBC, 273, 20383-20389 (1998). Peptides from the  $\alpha 10$  protein band were isolated by in-gel digestion with a trypsin and phase liquid chromatography and sequenced by Edman degradation (Camper et al, JBC, 273, 20383-20389 (1998).

#### Results

Fig 1 shows EDTA-eluted proteins from the fibronectin-Sepharose (A), flow-through from the collagen type II-Sepharose column (B) and EDTA-eluted proteins from the collagen type II-Sepharose (C). The  $\alpha 10$  integrin subunit (160 kDa) which was specifically eluted from the collagen type II column is indicated with an arrow. Figure 2 shows the amino acid sequences of six peptides that were isolated from the bovine integrin subunit  $\alpha 10$ . Figures 3 a, b, and c show that the  $\alpha 10$  integrin subunit is present on bovine chondrocytes (3a), human chondrocytes (3b) and human chondrosarcoma cells (3c). The affinity for collagen type II, the coprecipitation with  $\beta 1$ -integrin subunit and the molecular weight of 160 kDa under reducing conditions identify the  $\alpha 10$  integrin subunit on the different cells. These results show that  $\alpha 10$  can be isolated from chondrocytes and from chondrosarcoma cells.

#### Example 2

Amplification of PCR fragment corresponding to bovine  $\alpha 10$  integrin subunit.

#### Materials and methods

The degenerate primers GAY AAY ACI GCI CAR AC (DNTAQT, forward) and TIA TIS WRT GRT GIG GYT (EPHHSI, reverse) were used in PCR (Camper et al, JBC, 273, 20383-20389 (1998) to amplify the nucleotide sequence corresponding to the bovine peptide 1 (Figure 2). A 900 bp

PCR-fragment was then amplified from bovine cDNA using an internal specific primer TCA GCC TAC ATT CAG TAT (SAYIQY, forward) corresponding to the cloned nucleotide sequence of peptide 1 together with the degenerate primer ICK RTC CCA RTG ICC IGG (PGHWDR, reverse) corresponding to the bovine peptide 2 (Figure2). Mixed bases were used in positions that were twofold degenerate and inosines were used in positions that are three- or fourfold degenerate. mRNA isolation and cDNA synthesis was done as earlier described (Camper et al, JBC, 273, 20383-20389 (1998)). The purified fragment was cloned, purified and sequenced as earlier described (Camper et al, JBC, 273, 20383-20389 (1998)).

#### Results

The nucleotide sequence of peptide 1 (Figure 2) was obtained by PCR-amplification, cloning and sequencing of bovine cDNA. From this nucleotide sequence an exact primer was designed and applied in PCR-amplification with degenerate primers corresponding to peptides 2-6 (Figure 2). Primers corresponding to peptides 1 and 2 amplified a 900 bp PCR-fragment from bovine cDNA (Figure 4).

#### Example 3

Cloning and sequence analysis of the human  $\alpha 10$  integrin subunit

##### Material and methods

The cloned 900bp PCR-fragment, corresponding to bovine  $\alpha 10$ -integrin, was digoxigenin-labelled according to the DIG DNA labelling kit (Boehringer Mannheim) and used as a probe for screening of a human articular chondrocyte  $\lambda$ ZapII cDNA library (provided by Michael Bayliss, The Royal Veterinary Basic Sciences, London, UK) (52). Positive clones containing the pBluescript SK+ plasmid with the cDNA insert were rescued from the ZAP vector by *in vivo* excision as described in the ZAP-cDNA<sup>®</sup> synthesis kit (Stratagene). Selected plasmids were purified and

sequenced as described earlier (Camper et al, JBC, 273, 20383-20389 (1998)) using T3, T7 and internal specific primers. To obtain cDNA that encoded the 5' end of  $\alpha 10$  we designed the primer AAC TCG TCT TCC AGT GCC ATT CGT GGG (reverse; residue 1254-1280 in  $\alpha 10$  cDNA) and used it for rapid amplification of the cDNA 5' end (RACE) as described in the Marathon™ cDNA Amplification kit (Clontech INC., Palo Alto, CA).

#### Results

Two overlapping clones, hc1 and hc2 (Figure 5), were isolated, subcloned and sequenced. These clones contained 2/3 of the nucleotide sequence including the 3' end of the cDNA. A third clone (racel; Figure 5), which contained the 5' end of the  $\alpha 10$  cDNA, was obtained using the RACE technique. From these three overlapping clones of  $\alpha 10$  cDNA, 3884 nucleotides were sequenced. The nucleotide sequence and deduced amino acid sequence is shown in Figure 6. The sequence contains a 3504-nucleotide open reading frame that is predicted to encode a 1167 amino acid mature protein. The signal peptide cleavage site is marked with an arrow, human homologues to bovine peptide sequences are underlined and the I-domain is boxed. Metal ion binding sites are indicated with a broken underline, potential N-glycosylation sites are indicated by an asterisk and the putative transmembrane domain is double underlined. The normally conserved cytoplasmic sequence is indicated by a dot and dashed broken underline.

Sequence analysis demonstrate that  $\alpha 10$  is a member of the integrin  $\alpha$ -subunit family.

#### Example 4

Identification of a clone containing a splice variant of  $\alpha 10$

One clone which was isolated from the human chondrocyte library (see Example 3) contained a sequence that was identical to the sequence of  $\alpha 10$  integrin subunit except that the nucleotides between nt positions

2942 and 3055 were deleted. The splice variant of  $\alpha 10$  was verified in PCR experiment using primers flanking the splice region (see figure 14).

## 5 Example 5

Identification of  $\alpha 10$  integrin subunit by Northern blot

### Material and methods

Bovine chondrocyte mRNA was purified using a QuickPrep<sup>®</sup> Micro mRNA Purification Kit (Pharmacia Biotech, Uppsala, Sweden), separated on a 1% agarose-formaldehyde gel, transferred to nylon membranes and immobilised by UV crosslinking. cDNA-probes were <sup>32</sup>P-labelled with Random Primed DNA Labeling Kit (Boehringer Mannheim). Filters were prehybridised for 2-4 hours at 42°C in 5x SSE, 5x Denharts solution, 0.1 % SDS, 50 µg/ml salmon sperm DNA and 50% formamide and then hybridised over night at 42 °C with the same solution containing the specific probe (0.5-1 x 10<sup>6</sup> cpm/ml). Specifically bound cDNA-probes were analysed using the phosphoimager system (Fuji). Filters were stripped by washing in 0.1% SDS, for 1 hour at 80°C prior to re-probing. The  $\alpha 10$ -integrin cDNA-probe was isolated from the rac1-containing plasmid using the restriction enzymes BamHI (GIBCO BRL) and NcoI (Boehringer Mannheim). The rat  $\beta 1$ -integrin cDNA probe was a kind gift from Staffan Johansson, Uppsala, Sweden.

### Results

Northern blot analysis of mRNA from bovine chondrocytes showed that a human  $\alpha 10$  cDNA-probe hybridised with a single mRNA of approximately 5.4 kb (Figure 7). As a comparison, a cDNA-probe corresponding to the integrin subunit  $\alpha 1$  was used. This cDNA-probe hybridised a mRNA-band of approximately 3.5 kb on the same filter. These results show that a cDNA-probe against  $\alpha 10$  can be used to identify the  $\alpha 10$  integrin subunit on the mRNA level.



## Example 6

Preparation of antibodies against the integrin subunit  $\alpha 10$

A peptide corresponding to part of the  $\alpha 10$  cytoplasmic domain, Ckkipееееkreekle (see figure 6) was synthesised and conjugated to keyhole limpet hemocyanin (KLH). Rabbits were immunised with the peptide-KLH conjugate to generate antiserum against the integrin subunit  $\alpha 10$ . Antibodies recognising  $\alpha 10$  were affinity purified on an peptide-coupled column (Innovagen AB).

## Example 7

Immunoprecipitation of the integrin subunit  $\alpha 10$  from chondrocytes

## 15 Material and methods

Human chondrocytes were  $^{125}\text{I}$ -labelled, lysed with Triton X-100 and immunoprecipitated as earlier described (Holmvalle et al, Exp Cell Res, 221, 496-503 (1995), Camper et al, JBC, 273, 20383-20389 (1998)). Triton X-100 lysates of  $^{125}\text{I}$ -labeled human chondrocytes were immunoprecipitated with polyclonal antibodies against the integrin subunits  $\beta 1$ ,  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 3$  or  $\alpha 10$ . The immunoprecipitated proteins were separated by SDS-PAGE (4-12%) under non-reducing conditions and visualised using a phosphorimager. Triton X-100 lysates of human chondrocytes immunoprecipitated with  $\alpha 10$  or  $\beta 1$  were separated by SDS-PAGE (8%) under non-reducing conditions and analysed by Western blot using the polyclonal  $\beta 1$  antibody and chemiluminescent detection as described in Camper et al, JBC, 273, 20383-20389 (1998).

## Results

The polyclonal peptide antibody, raised against the cytoplasmic domain of  $\alpha 10$ , precipitated two protein bands with Mr of approximately 160 kD ( $\alpha 10$ ) and 125 kD ( $\beta 1$ ) under reducing conditions. The  $\alpha 10$  associated  $\beta$ -chain migrated as the  $\beta 1$  integrin subunit (Figure 8a). To verify that the  $\alpha 10$  associated  $\beta$ -chain in chondrocytes

indeed is  $\beta 1$ , chondrocyte lysates were immunoprecipitated with antibodies against  $\alpha 10$  or  $\beta 1$  followed by Western blot using antibodies against the  $\beta 1$ -subunit (Figure 8b). These results clearly demonstrated that  $\alpha 10$  is a member of the  $\beta 1$ -integrin family. However, the results do not exclude the possibility that  $\alpha 10$  can associate with other  $\beta$ -chains in other situations.

#### Example 8

10 Immunohistochemical staining of the integrin subunit  $\alpha 10$  in human and mouse cartilage

#### Material and methods

Frozen sections of adult cartilage (trochlear groove) obtained during surgery (provided by Anders Lindahl, Salgrenska Hospital, Gothenburg, Sweden and frozen sections from of 3 day old mouse limb were fixed and prepared for immunohistochemistry as earlier described (Camper et al, JBC, 273, 20383-20389 (1998)). Expression of  $\alpha 10$  integrin subunit was analysed using the polyclonal antibody against the cytoplasmic domain as a primary antibody (see Example 6) and a secondary antibody conjugated to peroxidase.

#### Results

Figures 9 show immunostaining of human adult articular cartilage.

The  $\alpha 10$ -antibody recognising the cytoplasmic domain of  $\alpha 10$  stained the chondrocytes in tissue sections of human articular cartilage (A). The staining was depleted when the antibody was preincubated with the  $\alpha 10$ - peptide (B). A control antibody recognising the  $\alpha 9$  integrin subunit did not bind to the chondrocyte (C).

Figures 10 shows that the  $\alpha 10$  antibody stain the majority of chondrocytes in the growing bone anlage (a and b). The  $\alpha 10$  antibody also recognised cells in the ossification groove of Ranvier (b), especially the osteoblast in the bone bark which are lining the cartilage in the metaphys are highly positive for  $\alpha 10$ . The

cells in the ossification groove of Ranvier are believed to be important for the growth in diameter of the bone. The integrin subunit  $\alpha 10$  is also highly expressed in perichondrium and periosteum. Cell in these tissues are likely important in the repair of the cartilage tissue. The described localisation of the integrin subunit  $\alpha 10$  suggest that this integrin is important for the function of the cartilage tissue.

#### 10 Example 9

Immunohistochemical staining of the integrin subunit  $\alpha 10$  during mouse development

##### Material and methods

Frozen sections from mouse embryos (13.5 days) were investigated for expression of  $\alpha 10$  by immunohistochemistry as described in Camper et al, JBC, 273, 20383-20389 (1998). Expression of  $\alpha 10$  integrin subunit was analysed using the polyclonal antibody against the cytoplasmic domain as a primary antibody (see Example 6) and a secondary antibody conjugated to peroxidase. The embryo sections were also investigated for expression of integrin subunit  $\alpha 1$  (monoclonal antibody from Pharmingen) and collagen type II (monoclonal antibody, kind gift from Dr John Mo, Lund University, Sweden).

#### 25 Results

Figure 11 show that  $\alpha 10$  integrin subunit is unregulated in the limb when the mesenchymal cells undergo condensation to form cartilage (a). Especially the edge of the newly formed cartilage has high expression of  $\alpha 10$ . The formation of cartilage is verified by the high expression of the cartilage specific collagen type II (b). The control antibody against  $\alpha 1$  integrin subunit showed only weak expression on the cartilage (c). In other experiments expression of  $\alpha 10$  was found in all cartilage containing tissues in the 3 day old mouse including limbs, ribs and vertebrae. The upregulation of  $\alpha 10$  during formation of cartilage suggest that this integrin subunit is

important both in the development of cartilage and bone and in the repair of damaged cartilage tissue.

#### Example 10

5 mRNA expression of  $\alpha 10$  in tissues other than articular cartilage

##### Material and methods

Expression of  $\alpha 10$  integrin subunit was examined on the mRNA level in different human tissues. A Northern dot blot with immobilised mRNA from the listed tissues in Figure 12 was hybridised with an  $\alpha 10$  integrin cDNA probe isolated from the race 1-containing plasmid using the restriction enzymes *Bam*H1 and *Nco*1. The degree of hybridisation was analysed using a phospho imager. The following symbols denote mRNA level in increasing order: -, +, ++, +++, +++++.

##### Results

Analysis of the hybridised mRNA showed that  $\alpha 10$  was expressed in aorta, trachea, spinal cord, heart, lung, and kidney (Figure 12). All other tissues appeared negative for  $\alpha 10$  expression. These results point to a restricted distribution of the  $\alpha 10$  integrin subunit.

#### Example 11

25 Immunohistochemical staining of  $\alpha 10$  in fascia around tendon and skeletal muscle and in tendon structures in heart valves.

##### Materials and methods

Frozen sections of adult cartilage (trochlear groove) obtained during surgery (provided by Anders Lindahl, Salgrenska Hospital, Gothenburg, Sweden and frozen sections from of 3 day old mouse limb were fixed and prepared for immunohistochemistry as earlier described (Camper et al, JBC, 273, 20383-20389 (1998)). Expression of  $\alpha 10$  integrin subunit was analysed using the polyclonal antibody against the cytoplasmic domain as a pri-

mary antibody (see Example 6) and a secondary antibody conjugated to peroxidase.

#### Results

As shown in figures 13 expression of  $\alpha 10$  was found  
5 in the fascia surrounding tendon (a) and skeletal muscle  
(b) and in the tendon structures in the heart valves (c).  
This localisation suggest that  $\alpha 10$  can bind to other  
matrix molecules in addition to the cartilage specific  
collagen type II. The localisation of the integrin  $\alpha 10$  on  
10 the surface of tendons indicate that  $\alpha 10$  can be involved  
in unwanted adhesion that often occurs between tendon/  
ligaments and the surrounding tissue after infection,  
injury or after surgery.

#### 15 Example 12

mRNA expression of  $\alpha 10$  integrin subunit in chondro-  
cytes, endothelial cells and fibroblasts.

#### Material and methods

Isolation of mRNA, synthesis of cDNA and PCR ampli-  
20 fication was done as earlier described (Camper et al,  
JBC, 273, 20383-20389 (1998)).

#### Results

Figure 14 shows PCR amplification of  $\alpha 10$  cDNA from  
human articular chondrocytes (lanes A6 and B1), human  
25 umbilical vein endothelial cells (lane A2), human fibro-  
blasts (lane A4) and rat tendon (Fig 14b, lane B2). Lanes  
1, 3, and 5 in figure 14 A show amplified fragments cor-  
responding to the integrin subunit  $\alpha 2$  in endothelial  
cells, fibroblasts and chondrocytes, respectively. cDNA-  
30 primers corresponding to the  $\alpha 10$  sequence positions nt  
2919-2943 (forward) and nt 3554-3578 (reverse) (see  
Figure 6) were used to amplify  $\alpha 10$  cDNA from the diffe-  
rent cells. The figure shows that  $\alpha 10$  was amplified in  
all three cell types. Two fragments of  $\alpha 10$  was amplified  
35 which represent the intact form of  $\alpha 10$  (larger fragment)  
and a splice variant (smaller fragment). The larger frag-

ment was dominating in chondrocytes while the smaller fragment was more pronounced in tendon (B2).

#### Example 13

5 Construction of  $\alpha 10$  mammalian expression vector.

The full length protein coding sequence of  $\alpha 10$  (combined from 3 clones, see figure 6) was inserted into the mammalian expression vector, pcDNA3.1/Zeo (Invitrogen). The vector contains SV40 promoter and Zeosin selection  
10 sequence. The  $\alpha 10$  containing expression vector was transfected into cells that express the  $\beta 1$ -integrin subunit but lack expression of the  $\alpha 10$  subunit. Expression of the  $\alpha 10$  integrin subunit on the cell surface can be analysed by immunoprecipitation and/or flow cytometry using anti-  
15 bodies specific for  $\alpha 10$ . The ligand binding capacity and the function of the inserted  $\alpha 10$  integrin subunit can be demonstrated in cell adhesion experiment and in signalling experiments.

#### 20 Example 14

Construction of mammalian expression vector containing a splice variant of  $\alpha 10$ .

The full length protein coding sequence of the splice variant of  $\alpha 10$  (nt 2942-nt3055 deleted) was  
25 inserted into the mammalian expression vector pcDNA3 (see Example 13). Expression and function of the splice variant can be analysed as described in example 13 and compared with the intact  $\alpha 10$  integrin subunit.

#### 30 Example 15

Partial isolation and characterisation of the  $\alpha 10$  integrin genomic DNA

Material and methods

Human  $\alpha 10$  cDNA, isolated from the racel-containing  
35 plasmid using the restriction enzymes *Bam*HI (GIBCO BRL) and *Nco*I (Boehringer Mannheim), was  $^{32}$ P-labelled and used as a probe for screening of a mouse 129 cosmid library

(provided by Reinhard Fässler, Lund University). Positive clones were isolated and subcloned. Selected plasmids were purified and sequenced as described earlier (Camper et al, JBC, 273, 20383-20389 (1998)) using T3, T7 and internal specific primers. Primers corresponding to mouse genomic DNA were then constructed and used in PCR to amplify and identify the genomic sequence of  $\alpha 10$  from the cosmid clones.

#### Results

Figure 15 shows 7958 nt of the  $\alpha 10$  gene. This partial genomic DNA sequence of  $\alpha 10$  integrin contains 8 exons, and a Kozak sequence. The mouse genomic  $\alpha 10$  sequence was used to generate a targeting vector for knockout experiments.

#### Example 16

Upregulation of  $\alpha 10$  integrin subunit in chondrocytes cultured in alginate beads

#### Material and methods

Human chondrocytes cultured in monolayer for 2 weeks were detached with trypsin-EDTA and introduced into alginate beads. Chondrocytes cultured in alginate are known to preserve their phenotype while chondrocytes cultured in monolayer are dedifferentiated. After 11 days chondrocytes cultured either in alginate or on monolayer were isolated and surface labelled with  $^{125}\text{I}$ . The  $\alpha 10$  integrin subunit was then immunoprecipitated with polyclonal antibodies recognising the cytoplasmic domain of  $\alpha 10$  (see Example 6 and Camper et al, JBC, 273, 20383-20389 (1998)).

#### Results

As shown in figure 16 chondrocytes cultured in alginate beads (lanes 3 and 4) upregulated their protein expression of  $\alpha 10\beta 1$ . This was in contrast to chondrocytes cultured in monolayer (lanes 1 and 2) which had a very low expression of  $\alpha 10\beta 1$ . Immunoprecipitation with ab control antibody is shown in lanes 1 and 3. It is known that

chondrocytes preserve their cartilage specific matrixpro-  
duction in alginate cultures but not in monolayer culture  
which point to that alginate preserve the phenotype of  
chondrocytes. These results support that  $\alpha 10$  integrin  
5 subunit can be used as a marker for differentiated chon-  
drocytes.

#### Example 17

Immunoprecipitation of the  $\alpha 10$  integrin subunit from  
10 human smooth muscle cells.

#### Material and methods

Human smooth muscle cells were isolated from human  
aorta. After one week in culture the cells were  $^{125}\text{I}$ -  
labelled, lysed and immunoprecipitated with antibodies  
15 against the integrin subunit  $\beta 1$  (lane 1),  $\alpha 1$  (lane 2),  $\alpha 2$   
(lane 3),  $\alpha 10$  (lane 4),  $\alpha 3$  (lane 5), control (lane 6)  
(Figure 17). The experiment was done as described in  
Example 7.

#### Results

20 The  $\alpha 10$  antibody precipitated two bands from the  
smooth muscle cells corresponding to the  $\alpha 10$  and the  $\beta 1$   
integrin subunit (Fig. 17).

#### Example 18

25 Construction of bacterial expression vector contain-  
ing sequence for  $\alpha 10$  splice region.

A plasmid for intracellular expression in E. coli  
of the alternatively spliced region (amino acid pos.  
952-986, SEQ. ID 1) was constructed as described. The  
30 alternatively spliced region were back-translated using  
the E. coli high frequency codon table, creating a cDNA  
sequence of 96% identity with the original sequence (SEQ.  
ID 1 nucleotide pos 2940-3044). Using sequence overlap  
extension (Horton et al., Biotechniques 8:528, 1990)  
35 primer  $\alpha 10\text{pfor}$  (tab. I) and  $\alpha 10\text{prev}$  (tab. I) was used  
to generate a double stranded fragment encoding the  $\alpha 10$   
amino acid sequence. This fragment was used as a PCR



template with primers  $\alpha 10$ pfor2 (tab. I) and  $\alpha 10$ prev2 (tab. I) in order to generate restriction enzyme site for sub-cloning in a pET vector containing the Z-domain of staphylococcal protein A, creating a fusion of the  $\alpha 10$  spliced region with the amino terminal of the Z-domain with trombin cleavage site residing in-between. The fragment generated in the second PCR reaction is shown (SEQ ID No. 3) also indicating the unique restriction enzymes used for sub-cloning in the expression vector.

10

Table I

$\alpha 10$ pfor	5' - G TTCAGAACCTGGGTTGCTACGTTGTTTCCGGTCTGATCATCTCCGC TCTGCTGCCGGCTGT-3'
$\alpha 10$ pfor2	5' -GGGGCATATGGTTCAGAACCTGGGTTGCTACGTTG-3'
$\alpha 10$ prev	5' - GATAACCTGGGACAAGCTTAGGAAGTAGTTACCACCGTGAGCAACAG CCGGCAGCAGAGCGGA-3'
$\alpha 10$ prev2	5' - GGGGGGATCCGCGCGGCACCAGGCCGCTGATAACCTGGGACAAGCTT AGGAAGT-3'

## REFERENCES

1. Springer, T.A. (1990) *Nature* **346**, 425-434
2. Ruoslahti, E. (1991) *J.Clin.Invest.* **87**, 1-5
3. Hynes, R.O. (1992) *Cell* **69**, 11-25
- 5 4. Hemler, M.E. (1988) *Immunol.Today* **9**, 109-113
5. Yamada, K.M. (1991) *J.Biol.Chem.* **266**, 12809-12812
6. Palmer, E.L., Ruegg, C., Ferrando, R., Pytela, R., and Sheppard, D. (1993) *J.Cell Biol.* **123**, 1289-1297
7. Takada, Y., Elices, M.J., Crouse, C., and Hemler, M.E. (1989) *EMBO J.* **8**, 1361-1368
- 10 8. Poncz, M., Eisman, R., Heidenreich, R., Silver, S.M., Vilaire, G., Surrey, S., Schwartz, E., and Bennett, J.S. (1987) *J.Biol.Chem.* **262**, 8476-8482
9. Larson, R.S., Corbi, A.L., Berman, L., and Springer, T. (1989) *J.Cell Biol.* **108**, 703-712
- 15 10. Corbi, A.L., Kishimoto, T.K., Miller, L.J., and Springer, T.A. (1988) *J.Biol.Chem.* **263**, 12403-12411
11. Argraves, W.S., Suzuki, S., Arai, H., Thompson, K., Pierschbacher, M.D., and Ruoslahti, E. (1987) *J.Cell Biol.* **105**, 1183-1190.
- 20 12. Corbi, A.L., Miller, L.J., O'Connor, K., Larson, R.S., and Springer, T.A. (1987) *EMBO J.* **6**, 4023-4028
13. Briesewitz, R., Epstein, M.R., and Marcantonio, E.E. (1993) *J.Biol.Chem.* **268**, 2989-2996
- 25 14. Ziober, B.L., Vu, M.P., Waleh, N., Crawford, J., Lin, C.S., and Kramer, R.H. (1993) *J.Biol.Chem.* **268**, 26773-26783
15. Hogervorst, F., Kuikman, I., van Kessel, A.G., and Sonnenberg, A. (1991) *Eur.J.Biochem.* **199**, 425-433
- 30 16. Takada, Y. and Hemler, M.E. (1989) *J.Cell Biol.* **109**, 397-407
17. Takada, Y., Murphy, E., Pil, P., Chen, C., Ginsberg, M.H., and Hemler, M.E. (1991) *J.Cell Biol.* **115**, 257-266
- 35 18. Van der Vieren, M., Le Trong, H., Wood, C.L., Moore, P.F., St.John, T., Staunton, D.E., and Gallatin, W.M. (1995) *Immunity.* **3**, 683-690

19. Schnapp, L.M., Breuss, J.M., Ramos, D.M., Sheppard, D., and Pytela, R. (1995) *J.Cell Sci.* 108, 537-544
20. Shaw, S.K., Cepek, K.L., Murphy, E.A., Russell, G.J., Brenner, M.B., and Parker, C.M. (1994) *J.Biol.Chem.* 269, 6016-6025
21. Suzuki, S., Argraves, W.S., Arai, H., Languino, L.R., Pierschbacher, M.D., and Ruoslahti, E. (1987) *J.Biol.Chem.* 262, 14080-14085
22. Ignatius, M.J., Large, T.H., Houde, M., Tawil, J.W., Barton, A., Esch, F., Carbonetto, S., and Reichardt, L.F. (1990) *J.Cell Biol.* 111, 709-720
23. Gullberg, D., Gehlsen, K.R., Turner, D.C., Åhlén, K., Zijenah, L.S., Barnes, M.J., and Rubin, K. (1992) *EMBO J.* 11, 3865-3873
24. Staaz, W.D., Rajpara, S.M., Wayner, E.A., Carter, W.G., and Santoro, S.A. (1989) *J.Cell Biol.* 108, 1917-1924
25. Holmval, K., Camper, L., Johansson, S., Rubin, K., Kimura, J.H., and Lundgren-Åkerlund, E. (1995) *Exp.Cell Res.* 221, 496-503
26. Forsberg, E., Ek, B., Engström, Å., and Johansson, S. (1994) *Exp.Cell Res.* 213, 183-190
27. Wayner, E.A. and Carter, W.G. (1987) *J.Cell Biol.* 105, 1873-1884
28. Weitzman, J.B., Pasqualini, R., Takada, Y., and Hemler, M.E. (1993) *J.Biol.Chem.* 268, 8651-8657
29. Elices, M.J. and Hemler, M.E. (1989) *Proc.Natl.Acad.Sci.U.S.A.* 86, 9906-9910
30. Languino, L.R., Colella, S., Zanetti, A., Andrieux, A., Ryckewaert, J.J., Charon, M.H., Marchisio, P.C., Plow, E.F., Ginsberg, M.H., Marguerie, G., and et al (1989) *Blood* 73, 734-742
31. Tuckwell, D.S., Humphries, M.J., and Brass, A. (1994) *Cell Adhes.Comm.* 2, 385-402
32. Springer, T.A. (1997) *Proc.Natl.Acad.Sci.U.S.A.* 94, 65-72

33. Colombatti, A., Bonaldo, P., and Doliana, R. (1993) Matrix 13, 297-306
34. Lee, C.H., Bradley, G., and Ling, V. (1995) Cell Growth Differ. 6, 347-354
- 5 35. Calderwood, D.A., Tuckwell, D.S., and Humphries, M.J. (1995) Biochem.Soc.Trans. 23, 504S
36. Kern, A., Eble, J., Golbik, R., and Kuhn, K. (1993) Eur.J.Biochem. 215, 151-159
37. Tuckwell, D.S., Reid, K.B., Barnes, M.J., and  
10 Humphries, M.J. (1996) Eur.J.Biochem. 241, 732-739
38. Kamata, T. and Takada, Y. (1994) J.Biol.Chem. 269, 26006-26010
39. Dürr, J., Goodman, S., Potocnik, A., von der Mark, H., and von der Mark, K. (1993) Exp.Cell Res. 207,  
15 235-244
40. Salter, D.M., Hughes, D.E., Simpson, R., and Gardner, D.L. (1992) Br.J.Rheumatol. 31, 231-234
41. Woods, V.L.J., Schreck, P.J., Gesink, D.S., Pacheco, H.O., Amiel, D., Akeson, W.H., and Lotz, M. (1994)  
20 Arthritis Rheum. 37, 537-544
42. Camper, L., Heinegård, D., and Lundgren-Åkerlund, E. (1997) J.Cell Biol 138, 1159-1167
43. Hemler, M.E., Sanchez Madrid, F., Flotte, T.J., Krensky, A.M., Burakoff, S.J., Bhan, A.K., Springer, T.A., and Strominger, J.L. (1984) J.Immunol. 132,  
25 3011-3018
44. Bottger, B.A., Hedin, U., Johansson, S., and Thyberg, J. (1989) Differentiation. 41, 158-167
45. Sommarin, Y. and Heinegård, D. (1983) Biochem.J. 214,  
30 777-784
46. Häuselmann, H.J., Aydelotte, M.B., Schumacher, B.L., Kuettner, K.E., Gitelis, S.H., and Thonar, E.J.M.A. (1992) Matrix 12, 116-129
47. Miller, E.J. (1972) Biochemistry 11, 4903-4909
- 35 48. Wessel, D. and Flugge, U.I. (1984) Anal.Biochem. 138, 141-143

49. Blobel, G. and Dobberstein, B. (1975) *J.Cell Biol.* 67, 835-851
50. Hellman, U. (1997) in *Protein structure analysis. Preparation, characterization, and microsequencing* (Kamp, R.M., Choli-Papadopoulou, T., and Wittmann-Liebold, B., eds) pp. 97-104, Springer-Verlag, Heidelberg
51. Charles, I.G., Palmer, R.M., Hickery, M.S., Bayliss, M.T., Chubb, A.P., Hall, V.S., Moss, D.W., and Moncada, S. (1993) *Proc.Natl.Acad.Sci.U.S.A.* 90, 11419-11423
52. Tuckwell, D.S., Brass, A., and Humphries, M.J. (1992) *Biochem.J.* 285, 325-331
53. Dedhar, S. and Hannigan, G.E. (1996) *Curr.Opin.Cell Biol.* 8, 657-669
54. Hughes, P.E., O'Toole, T.E., Ylanne, J., Shattil, S.J., and Ginsberg, M.H. (1995) *J.Biol.Chem.* 270, 12411-12417
55. Puzon McLaughlin, W., Yednock, T.A., and Takada, Y. (1996) *J.Biol.Chem.* 271, 16580-16585
56. O'Toole, T.E., Katagiri, Y., Faull, R.J., Peter, K., Tamura, R., Quaranta, V., Loftus, J.C., Shattil, S.J., and Ginsberg, M.H. (1994) *J.Cell Biol.* 124, 1047-1059
57. De Melker, A.A., Kramer, D., Kuikman, I., and Sonnenberg, A. (1997) *Biochem J* 529-537
58. Rojiani, M.V., Finlay, B.B., Gray, V., and Dedhar, S. (1991) *Biochemistry* 30, 9859-9866
59. Coppolino, M.G., Woodside, M.J., Demaurex, N., Grinstein, S., St Arnaud, R., and Dedhar, S. (1997) *Nature* 386, 843-847
60. Hynes, R.O. (1992) *Curr.Opin.Genet.Dev.* 2, 621-624
61. Santoro, S.A. (1986) *Cell* 46, 913-920
62. Languino, L.R., Gehlsen, K.R., Wayner, E., Carter, W.G., Engvall, E., and Ruoslahti, E. (1989) *J.Cell Biol.* 109, 2455-2462

63. Yokosaki, Y., Monis, H., Chen, J., and Sheppard, D.  
(1996) J.Biol.Chem. 271, 24144-24150

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) NUMBER OF SEQUENCES: 2

## (2) INFORMATION FOR SEQ ID NO. 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3884 base pairs  
(B) TYPE: nucleic acid and amino acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (E) ORGANISM: human  
(F) CELLTYPE: chondrocyte

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 1:

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1  -----+-----+-----+-----+-----+-----+-----+ 60
GTCCAGTCTTTGGCTAGTCCGTACCTTGAGGGGAAGCAGTGAGTGGACAAGAACGGGGAC

a      M E L P F V T H L F L P L -

GTGTTCTGACAGGTCTCTGCTCCCCCTTTAACCTGGATGAACATCACCCACGCCTATTC
61  -----+-----+-----+-----+-----+-----+-----+ 120
CACAAGGACTGTCCAGAGACGAGGGGGAATTTGGACCTACTTGTAGTGGGTGCGGATAAG

a      V F L T G L C S P F N L D E H H P R L F -

CCAGGGCCACCAGAAGCTGAATTTGATACAGTGTCTTACAACATGTTGGGGGTGGACAG
121  -----+-----+-----+-----+-----+-----+-----+ 180
GGTCCCGGTGGTCTTCGACTTAAACCTATGTCACAGAATGTTGTACAACCCCCACCTGTC

a      P G P P E A E F G Y S V L Q H V G G G Q -

CGATGGATGCTGGTGGGCGCCCCCTGGGATGGGCCTTCAGGCGACCGGAGGGGGGACGTT
181  -----+-----+-----+-----+-----+-----+-----+ 240
GCTACCTACGACCACCCGCGGGGACCCCTACCGGAAGTCCGCTGGCCTCCCCCTGCAA

a      R W M L V G A P W D G P S G D R R G D V -

TATCGCTGCCCTGTAGGGGGGGCCCAATGCCCCATGTGCAAGGGCCACTTAGGTGAC
241  -----+-----+-----+-----+-----+-----+-----+ 300
ATAGCGACGGGACATCCCCCGGGTGTACGGGGTACACGGTTCCCGGTGAATCCACTG

a      Y R C P V G G A H N A P C A K G H L G D -

TACCAACTGGGAAATTCATCTCATCCTGCTGTGAATATGCACCTGGGGATGTCTCTGTTA
301  -----+-----+-----+-----+-----+-----+-----+ 360
ATGGTTGACCCCTTTAAGTAGAGTAGGACGACACTTATACGTGGACCCCTACAGAGACAAT

a      Y Q L G N S S H P A V N M H L G M S L L -

GAGACAGATGGTGATGGGGGATTTCATGGCCTGTGCCCTCTCTGGTCTCGTGCTTGTGGC
361  -----+-----+-----+-----+-----+-----+-----+ 420
CTCTGTCTACCACTACCCCTAAGTACCGGACACGGGGAGAGACCAGAGCACGAACACCG

a      E T D G D G G F M A C A P L W S R A C G -
```

AGCTCTGTCTTCAGTTCTGGGATATGTGCCCGTGTGGATGCTTCATTCCAGCCTCAGGGA  
421 -----+-----+-----+-----+-----+-----+ 480  
TCGAGACAGAAGTCAAGACCCTATACACGGGCACACCTACGAAGTAAGGTCGGAGTCCCT

a S S V F S S G I C A R V D A S F Q P Q G -

AGCCTGGCACCCACTGCCCAACGCTGCCCAACATACATGGATGTTGTCTTGTCTTGGAT  
481 -----+-----+-----+-----+-----+-----+ 540  
TCGGACCGTGGGTGACGGGTTGCGACGGGTTGTATGTACCTACAACAGTAACAGAACCTA

a S L A P T A Q R C P T Y M D V V I V L D -

GGCTCCAACAGCATCTACCCCTGGTCTGAAGTTCAGACCTTCCTACGAAGACTGGTAGGG  
541 -----+-----+-----+-----+-----+-----+ 600  
CCGAGGTTGTCTAGATGGGGACCAGACTTCAAGTCTGGAAGGATGCTTCTGACCATCCC

a G S N S I Y P W S E V Q T F L R R L V G -

AAACTGTTTATTGACCCAGAACAGATACAGGTGGGACTGGTACAGTATGGGGAGAGCCCT  
601 -----+-----+-----+-----+-----+-----+ 660  
TTTGACAAATAACTGGGTCTTGTCTATGTCCACCCTGACCATGTCATACCCCTCTCGGGA

a K L F I D P E Q I Q V G L V Q Y G E S P -

GTACATGAGTGGTCCCTGGGAGATTTCGGAACGAAGGAAGAAGTGGTGAGAGCAGCAAAG  
661 -----+-----+-----+-----+-----+-----+ 720  
CATGTACTCACCAGGACCCTCTAAAGGCTTGCTTCCTTCTCACCCTCTCGTCTGTTTC

a V H E W S L G D F R T K E E V V R A A K -

AACCTCAGTCGGCGGGAGGGACGAGAAACAAAGACTGCCCAAGCAATAATGGTGGCCTGC  
721 -----+-----+-----+-----+-----+-----+ 780  
TTGGAGTCAGCCGCCCTCCCTGCTCTTTGTTTCTGACGGGTTGCTTATTACCACCGGACG

a N L S R R E G R E T K T A Q A I M V A C -

ACAGAAGGGTTCAGTCAGTCCCATGGGGGCCGACCCGAGGCTGCCAGGCTACTGGTGGTT  
781 -----+-----+-----+-----+-----+-----+ 840  
TGTCTTCCCAAGTCAGTCAGGGTACCCCCGGCTGGGCTCCGACGGTCCGATGACCACCAA

a T E G F S Q S H G G R P E A A R L L V V -

GTCATGATGGAGAGTCCCATGATGGAGAGGAGCTTCCTGCAGCACTAAAGGCCTGTGAG  
841 -----+-----+-----+-----+-----+-----+ 900  
CAGTGACTACCTCTCAGGGTACTACCTCTCCTCGAAGGACGTCGTGATTTCCGGACACTC

a V T D G E S H D G E E L P A A L K A C E -

GCTGGAAGAGTGACACGCTATGGGATTGCAGTCCTTGGTCACTACCTCCGGCGGCAGCGA  
901 -----+-----+-----+-----+-----+-----+ 960  
CGACCTTCTCACTGTGCGATACCCTAACGTCAGGAACAGTGATGGAGGCCGCCGTCGCT

a A G R V T R Y G I A V L G H Y L R R Q R -

GATCCCAGCTCTTTCTGAGAGAAATTAGAACTATTGCCAGTGATCCAGATGAGCGATTTC  
961 -----+-----+-----+-----+-----+-----+ 1020  
CTAGGTCGAGAAAGGACTCTCTTTAATCTTGATAACGGTCACTAGGTCTACTCGCTAAG

a D P S S F L R E I R T I A S D P D E R F -

TTCTTCAATGTACAGATGAGGCTGCTCTGACTGACATTGTGGATGCACTAGGAGATCGG  
1021 -----+-----+-----+-----+-----+-----+ 1080  
AAGAAGTTACAGTGTCTACTCCGACGAGACTGACTGTAACACCTACGTGATCCTCTAGCC

a F F N V T D E A A L T D I V D A L G D R -



1081 ATTTTGGCCTTGAAGGGTCCCATGCAGAAAACGAAAGCTCCTTTGGGCTGGAAATGTCT 1140  
-----+-----+-----+-----+-----+-----+  
TAAAAACCGGAACCTCCAGGGTACGTCTTTTGCTTTCGAGGAAACCCGACCTTTACAGA

a I F G L E G S H A E N E S S F G L E M S -

1141 CAGATTGGTTTCTCCACTCATCGGCTAAAGGATGGGATTCTTTTGGGATGGTGGGGGCC  
-----+-----+-----+-----+-----+-----+ 1200  
GTCTAACCAAAGAGGTGAGTAGCCGATTTCCTACCTAAGAAAAACCTACCACCCCCGG

a Q I G F S T H R L K D G I L F G M V G A -

1201 TATGACTGGGGAGGCTCTGTGCTATGGCTTGAAGGAGGCCACCGCCTTTTCCCCCACGA  
-----+-----+-----+-----+-----+-----+ 1260  
ATACTGACCCCTCCGAGACACGATACCGAACTTCCTCCGGTGGCGGAAAGGGGGGTGCT

a Y D W G G S V L W L E G G H R L F P P R -

1261 ATGGCACTGGAAGACGAGTTCCCCCTGCACTGCAGAACCATGCAGCCTACCTGGGTAC  
-----+-----+-----+-----+-----+-----+ 1320  
TACCGTGACCTTCTGCTCAAGGGGGGACGTGACGTCTTGGTACGTGCGATGGACCCAATG

a M A L E D E F P P A L Q N H A A Y L G Y -

1321 TCTGTTTCTTCCATGCTTTTGGGGGTGGACGCCGCTGTTCTCTCTGGGGCTCCTCGA  
-----+-----+-----+-----+-----+-----+ 1380  
AGACAAAGAAGGTACGAAAACGCCCCACCTGCGGCGGACAAAGAGAGACCCCGAGGAGCT

a S V S S M L L R G G R R L F L S G A P R -

1381 TTTAGACATCGAGGAAAAGTCATCGCCTTCCAGCTTAAGAAAGATGGGGCTGTGAGGGTT  
-----+-----+-----+-----+-----+-----+ 1440  
AAATCTGTAGCTCCTTTTCAGTAGCGGAAGGTGCAATTCTTTCTACCCCGACACTCCCAA

a F R H R G K V I A F Q L K K D G A V R V -

1441 GCCCAGAGCCTCCAGGGGAGCAGATTGGTTCATACTTGGCAGTGAGCTCTGCCCATTG  
-----+-----+-----+-----+-----+-----+ 1500  
CGGGTCTCGGAGGTCCCCCTCGTCTAACCAAGTATGAAACCGTCACTCGAGACGGGTAAC

a A Q S L Q G E Q I G S Y F G S E L C P L -

1501 GATACAGATAGGGATGGAACAACTGATGTCTTACTTGTGGCTGCCCCATGTTCTTGGGA  
-----+-----+-----+-----+-----+-----+ 1560  
CTATGTCTATCCCTACCTTGTGACTACAGAATGAACACCGACGGGGGTACAAGGACCCT

a D T D R D G T T D V L L V A A P M F L G -

1561 CCCCAGAACAAGGAAACAGGACGTGTTTATGTGTATCTGGTAGGCCAGCAGTCCTTGCTG  
-----+-----+-----+-----+-----+-----+ 1620  
GGGGTCTGTTCTTGTCTGTCACAAATACATAGACCATCCGGTCGTCAGGAACGAC

a P Q N K E T G R V Y V Y L V G Q Q S L L -

1621 ACCCTCCAAGGAACACTTCAGCCAGAACCCCCCAGGATGCTCGGTTTGGCTTTGCCATG  
-----+-----+-----+-----+-----+-----+ 1680  
TGGGAGGTTCTTGTGAAGTCGGTCTTGGGGGGTCTACGAGCCAAACCGAAACGGTAC

a T L Q G T L Q P E P P Q D A R F G F A M -

1681 GGAGCTCTTCTGATCTGAACCAAGATGGTTTTGCTGATGTGGCTGTGGGGGCGCCTCTG  
-----+-----+-----+-----+-----+-----+ 1740  
CCTCGAGAAGGACTAGACTTGGTTCTACAAAACGACTACACCGACACCCCCGCGGAGAC

a G A L P D L N Q D G F A D V A V G A P L -

GAAGATGGGCACCAGGGAGCACTGTACCTGTACCATGGAACCCAGAGTGGAGTCAGGCCC  
1741 -----+-----+-----+-----+-----+ 1800  
CTTCTACCCGTGGTCCCTCGTGACATGGACATGGTACCTTGGGTCTCACCTCAGTCCGGG

a E D G H Q G A L Y L Y H G T Q S G V R P -

CATCCTGCCCAGAGGATTGCTGCTGCCTCCATGCCACATGCCCTCAGCTACTTTGGCCGA  
1801 -----+-----+-----+-----+-----+ 1860  
GTAGGACGGGTCTCCTAACGACGACGGAGGTACGGTGTACGGGAGTCGATGAAACCGGCT

a H P A Q R I A A A S M P H A L S Y F G R -

AGTGTGGATGGTCGGCTAGATCTGGATGGAGATGATCTGGTCGATGTGGCTGTGGGTGCC  
1861 -----+-----+-----+-----+-----+ 1920  
TCACACCTACCAGCCGATCTAGACCTACCTCTACTAGACCAGCTACACCGACACCCACGG

a S V D G R L D L D G D D L V D V A V G A -

CAGGGGGCAGCCATCCTGCTCAGCTCCCGGCCATTGTCCATCTGACCCCATCACTGGAG  
1921 -----+-----+-----+-----+-----+ 1980  
GTCCCCCGTCGGTAGGACGAGTCGAGGGCCGGTAACAGGTAGACTGGGGTAGTGACCTC

a Q G A A I L L S S R P I V H L T P S L E -

GTGACCCACAGGCCATCAGTGTGGTTCAGAGGGACTGTAGGCGGCGAGGCCAAGAAGCA  
1981 -----+-----+-----+-----+-----+ 2040  
CACTGGGGTGTCCGGTAGTCACACCAAGTCTCCCTGACATCCGCCGCTCCGGTTCTTCGT

a V T P Q A I S V V Q R D C R R R G Q E A -

GTCTGTCTGACTGCAGCCCTTTGCTTCCAAGTGACCTCCCGTACTCCTGGTCGCTGGGAT  
2041 -----+-----+-----+-----+-----+ 2100  
CAGACAGACTGACGTCCGGAAACGAAGGTTCACTGGAGGGCATGAGGACCAGCGACCCTA

a V C L T A A L C F Q V T S R T P G R W D -

CACCAATTCTACATGAGGTTACCGCATCACTGGATGAATGGACTGCTGGGGCACGTGCA  
2101 -----+-----+-----+-----+-----+ 2160  
GTGGTTAAGATGTACTCCAAGTGGCGTAGTGACCTACTTACCTGACGACCCCGTGCACGT

a H Q F Y M R F T A S L D E W T A G A R A -

GCATTTGATGGCTCTGGCCAGAGGTTGTCCCTCGGAGGCTCCGGCTCAGTGTGGGGAAT  
2161 -----+-----+-----+-----+-----+ 2220  
CGTAAACTACCGAGACCGGTCTCCAACAGGGGAGCCTCCGAGGCCGAGTCACACCCCTTA

a A F D G S G Q R L S P R R L R L S V G N -

GTCACCTGTGAGCAGCTACACTTCCATGTGCTGGATACATCAGATTACCTCCGGCCAGTG  
2221 -----+-----+-----+-----+-----+ 2280  
CAGTGAACACTCGTCGATGTGAAGGTACACGACCTATGTAGTCTAATGGAGGCCGGTCAC

a V T C E Q L H F H V L D T S D Y L R P V -

GCCTTGACTGTGACCTTTGCCTTGGACAATACTACAAAGCCAGGGCCTGTGCTGAATGAG  
2281 -----+-----+-----+-----+-----+ 2340  
CGGAACTGACACTGGAACCGGAACCTGTATGATGTTTCGGTCCCGGACACGACTTACTC

a A L T V T F A L D N T T K P G P V L N E -

GGCTCACCCACCTCTATACAAAAGCTGGTCCCCTTCTCAAAGGATTGTGGCCCTGACAA  
2341 -----+-----+-----+-----+-----+ 2400  
CCGAGTGGGTGGAGATATGTTTCGACCAGGGGAAGAGTTTCCTAACACCGGGACTGTTA

a G S P T S I Q K L V P F S K D C G P D N -

GAATGTGTCACAGACCTGGTGCTTCAAGTGAATATGGACATCAGAGGCTCCAGGAAGGCC  
2401 -----+-----+-----+-----+-----+-----+ 2460  
CTTACACAGTGTCTGGACCACGAAGTTCACCTTATACCTGTAGTCTCCGAGGTCCTTCCGG

a E C V T D L V L Q V N M D I R G S R K A -

CCATTTGTGGTTCGAGGTGGCCGGCGAAAGTGTGGTATCTACAACCTCTGGAGAACAGA  
2461 -----+-----+-----+-----+-----+-----+ 2520  
GGTAAACACCAAGCTCCACCGCCGCTTTCACGACCATAGATGTTGAGACCTCTTGTCT

a P F V V R G G R R K V L V S T T L E N R -

AAGGAAAATGCTTACAATACGAGCCTGAGTATCATCTTCTCTAGAAACCTCCACCTGGCC  
2521 -----+-----+-----+-----+-----+-----+ 2580  
TTCCTTTTACGAATGTTATGCTCGGACTCATAGTAGAAGAGATCTTTGGAGGTGGACCGG

a K E N A Y N T S L S I I F S R N L H L A -

AGTCTCACTCCTCAGAGAGAGAGCCCAATAAAGGTGGAATGTGCCGCCCTTCTGCTCAT  
2581 -----+-----+-----+-----+-----+-----+ 2640  
TCAGAGTGAGGAGTCTCTCTCTCGGTTATTTCCACCTTACACGGCGGGGAAGACGAGTA

a S L T P Q R E S P I K V E C A A P S A H -

GCCCGGCTCTGCAGTGTGGGCATCCTGTCTTCCAGACTGGAGCCAAGGTGACCTTTCTG  
2641 -----+-----+-----+-----+-----+-----+ 2700  
CGGGCCGAGACGTACACCCCGTAGGACAGAAGGTCTGACCTCGGTTCCACTGGAAAGAC

a A R L C S V G H P V F Q T G A K V T F L -

CTAGAGTTTGAGTTTAGCTGCTCCTCTCTCCTGAGCCAGGTCTTTGGGAAGCTGACTGCC  
2701 -----+-----+-----+-----+-----+-----+ 2760  
GATCTCAAACCTCAAATCGACGAGGAGAGAGGACTCGGTCCAGAAACCTTCGACTGACGG

a L E F E F S C S S L L S Q V F G K L T A -

AGCAGTGACAGCCTGGAGAGAAATGGCACCCTTCAAGAAAACACAGCCCAGACCTCAGCC  
2761 -----+-----+-----+-----+-----+-----+ 2820  
TCGTCACTGTGGACCTCTCTTTACCGTGGGAAGTTCTTTTGTGTCGGGTCTGGAGTCGG

a S S D S L E R N G T L Q E N T A Q T S A -

TACATCCAATATGAGCCCCACCTCCTGTTCTCTAGTGAGTCTACCCTGCACCGCTATGAG  
2821 -----+-----+-----+-----+-----+-----+ 2880  
ATGTAGGTTATACTCGGGGTGGAGGACAAGAGATCACTCAGATGGGACGTGGCGATACTC

a Y I Q Y E P H L L F S S E S T L H R Y E -

GTTCACCCATATGGGACCCTCCCAGTGGGTCTGGCCCAGAATTCAAACCACTCTCAGG  
2881 -----+-----+-----+-----+-----+-----+ 2940  
CAAGTGGGTATACCCTGGGAGGGTCACCCAGGACCGGTCTTAAGTTTGGTGAGAGTCC

a V H P Y G T L P V G P G P E F K T T L R -

GTTCAGAACCTAGGCTGTATGTGGTCAGTGGCCTCATCATCTCAGCCCTCCTTCCAGCT  
2941 -----+-----+-----+-----+-----+-----+ 3000  
CAAGTCTTGATCCGACGATACACAGTCACCGGAGTAGTAGAGTCGGGAGGAAGGTCTGA

a V Q N L G C Y V V S G L I I S A L L P A -

GTGGCCCATGGGGCAATTACTTCTATCACTGTCTCAAGTCATCACTAACAATGCAAGC  
3001 -----+-----+-----+-----+-----+-----+ 3060  
CACCGGTACCCCCGTTAATGAAGGATAGTGACAGAGTTCAGTAGTGATTGTTACGTTCCG

a V A H G G N Y F L S L S Q V I T N N A S -

3061 TGCATAGTGCAGAACCTGACTGAACCCCCAGGCCACCTGTGCATCCAGAGGAGCTTCAA  
-----+-----+-----+-----+-----+ 3120  
ACGTATCACGTCTTGGACTGACTTGGGGGTCCGGGTGGACACGTAGGTCTCCTCGAAGTT

a C I V Q N L T E P P G P P V H P E E L Q -

3121 CACACAAACAGACTGAATGGGAGCAATACTCAGTGTGAGGTGGTGGAGGTGCCACCTTGGG  
-----+-----+-----+-----+-----+ 3180  
GTGTGTTTGTCTGACTTACCCTCGTTATGAGTCACAGTCCACCACTCCACGGTGGAAACC

a H T N R L N G S N T Q C Q V V R C H L G -

3181 CAGCTGGCAAAGGGGACTGAGGTCTCTGTTGGACTATTGAGGCTGGTTCACAATGAATTT  
-----+-----+-----+-----+-----+ 3240  
GTCGACCGTTTCCCCTGACTCCAGAGACAACCTGATAACTCCGACCAAGTGTACTTAAA

a Q L A K G T E V S V G L L R L V H N E F -

3241 TTCCGAAGAGCCAAGTTCAAGTCCCTGACGGTGGTCCAGCACCTTTGAGCTGGGAACCGAA  
-----+-----+-----+-----+-----+ 3300  
AAGGCTTCTCGGTTCAAGTTCAGGGACTGCCACCACTCGTGGAACTCGACCCCTGGCTT

a F R R A K F K S L T V V S T F E L G T E -

3301 GAGGGCAGTGTCTACAGCTGACTGAAGCCTCCCGTTGGAGTGAGAGCCTCTTGAGGTG  
-----+-----+-----+-----+-----+ 3360  
CTCCCGTCACAGGATGTCGACTGACTTCGGAGGGCAACCTCACTCTCGGAGAACCTCCAC

a E G S V L Q L T E A S R W S E S L L E V -

3361 GTTCAGACCCGGCCTATCCTCATCTCCCTGTGGATCCTCATAGGCAGTGTCTGGGAGGG  
-----+-----+-----+-----+-----+ 3420  
CAAGTCTGGGCGGATAGGAGTAGAGGGACACCTAGGAGTATCCGTACAGGACCCCTCC

a V Q T R P I L I S L W I L I G S V L G G -

3421 TTGCTCCTGCTTGCTCTCCTTGTCTTCTGCCTGTGGAAGCTTGGCTTCTTTGCCATAAG  
-----+-----+-----+-----+-----+ 3480  
AACGAGGACGAACGAGAGGAACAGAAGACGGACACCTTCGAACGAAGAAACGGGTATTC

a L L L L A L L V F C L W K L G F F A H K -

3481 AAAATCCCTGAGGAAGAAAAAGAGAAGAGAAGTTGGAGCAATGAATGTAGAATAAGGGT  
-----+-----+-----+-----+-----+ 3540  
TTTTAGGGACTCCTTCTTTTCTCTTCTTCAACCTCGTTACTTACATCTTATTTCCA

a K I P E E E K R E E K L E Q

3541 CTAGAAAGTCCCTCCCTGGCAGCTTTCTTCAAGAGACTTGCATAAAAGCAGAGGTTTGGGG  
-----+-----+-----+-----+-----+ 3600  
GATCTTTCAGGAGGGACCGTCGAAAGAAGTTCTCTGAACGTATTTTCGTCTCCAACCCC

3601 GCTCAGATGGGACAAGAAGCCGCCTCTGGACTATCTCCCCAGACCAGCAGCCTGACTTGA  
-----+-----+-----+-----+-----+ 3660  
CGAGTCTACCCTGTTCTTCGGCGGAGACCTGATAGAGGGGTCTGGTCGTCGGACTGAACT

3661 CTTTTGAGTCCTAGGGATGCTGCTGGCTAGAGATGAGGCTTTACCTCAGACAAGAAGAGC  
-----+-----+-----+-----+-----+ 3720  
GAAAACTCAGGATCCCTACGACGACCGATCTCTACTCCGAAATGGAGTCTGTTCTTCTCG

3721 TGGCACCAAACTAGCCATGCTCCCACCCTCTGCTTCCCTCCTCCTCGTGATCCTGGTTC  
-----+-----+-----+-----+-----+-----+ 3780  
ACCGTGGTTTGTATCGGTACGAGGGTGGGAGACGAAGGGAGGAGGAGCACTAGGACCAAG

3781 CATAGCCAACACTGGGGCTTTTGTGTTGGGGTCCTTTTATCCCCAGGAATCAATAATTTT  
-----+-----+-----+-----+-----+-----+ 3840  
GTATCGGTTGTGACCCCGAAAACAAACCCAGGAAAATAGGGGTCCTTAGTTATTAAAAA

3841 TTGCCTAGGAAAAAAAAAAGCGGCCGCGAATTCGATATCAAGCT  
-----+-----+-----+-----+-----+ 3884  
AACGGATCCTTTTTTTTTTCGCCGGCGCTTAAGCTATAGTTCGA

43

## (2) INFORMATION FOR SEQ ID NO. 2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3779 base pairs
- (B) TYPE: nucleic acid and amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- (E)

## (i) MOLECULAR TYPE: cDNA

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: human
- (B) CELLTYPE: chondrocyte

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO. 2:

```
CAGGTCAGAAACCGATCAGGCATGGAACCTCCCTTCGTCACCTCACCTGTTCTTGCCCCCTG
1  -----+-----+-----+-----+-----+-----+ 60
GTCCAGTCTTTGGCTAGTCCGTACCTTGAGGGGAAGCAGTGAGTGGACAAGAACGGGGAC

          M E L P F V T H L F L P L -

GTGTTCTGACAGGTCTCTGCTCCCCCTTTAACCTGGATGAACATCACCCACGCCTATTC
61  -----+-----+-----+-----+-----+-----+ 120
CACAAAGGACTGTCCAGAGACGAGGGGGAAATTGGACCTACTTGTAGTGGGTGCGGATAAG

a      V F L T G L C S P F N L D E H H P R L F -

CCAGGGCCACCAGAAGCTGAATTTGGATACAGTGTCTTACAACATGTTGGGGGTGGACAG
121 -----+-----+-----+-----+-----+-----+ 180
GGTCCCGGTGGTCTTCGACTTAAACCTATGTCACAGAATGTTGTACAACCCCCACCTGTC

a      P G P P E A E F G Y S V L Q H V G G G Q -

CGATGGATGCTGGTGGGCGCCCCCTGGGATGGGCCTTCAGGCGACCGAGGGGGACGTT
181 -----+-----+-----+-----+-----+-----+ 240
GCTACCTACGACACCCCGGGGGGACCCTACCCGGAAGTCCGCTGGCCTCCCCCTGCAA

a      R W M L V G A P W D G P S G D R R G D V -

TATCGCTGCCCTGTAGGGGGGGCCACAAATGCCCCATGTGCCAAGGGCCACTTAGGTGAC
241 -----+-----+-----+-----+-----+-----+ 300
ATAGCGACGGGACATCCCCCGGGGTGTTACGGGGTACACGGTTCCCGGTGAATCCACTG

a      Y R C P V G G A H N A P C A K G H L G D -

TACCAACTGGGAAATTCATCTCATCCTGCTGTGAATATGCACCTGGGGATGTCTCTGTTA
301 -----+-----+-----+-----+-----+-----+ 360
ATGGTTGACCCTTTAAGTAGAGTAGGACGACACTTATACGTGGACCCCTACAGAGACAAT

a      Y Q L G N S S H P A V N M H L G M S L L -

GAGACAGATGGTGATGGGGGATTTCATGGCCTGTGCCCCCTCTCTGGTCTCGTGCTTGTGGC
361 -----+-----+-----+-----+-----+-----+ 420
CTCTGTCTACCACTACCCCTAAGTACCGGACACGGGGAGAGACCAGAGCACGAACACCG

a      E T D G D G G F M A C A P L W S R A C G -

AGCTCTGTCTTCAGTTCTGGGATATGTGCCCGTGTGGATGCTTCATTCCAGCCTCAGGGA
421 -----+-----+-----+-----+-----+-----+ 480
TCGAGACAGAAGTCAAGACCCCTATACACGGGCACACCTACGAAGTAAGGTCGGAGTCCTT
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44

a S S V F S S G I C A R V D A S F Q P Q G -  
AGCCTGGCACCCTGCCCCACGCTGCCAACATACATGGATGTTGTCATTGTCTTGGAT  
481 -----+-----+-----+-----+-----+ 540  
TCGGACCGTGGGTGACGGGTGCGACGGGTGTATGTACCTACAACAGTAACAGAACCTA

a S L A P T A Q R C P T Y M D V V I V L D -  
GGCTCCAACAGCATCTACCCCTGGTCTGAAGTTCAGACCTTCCTACGAAGACTGGTAGGG  
541 -----+-----+-----+-----+-----+ 600  
CCGAGGTTGTCTAGATGGGGACAGACTTCAAGTCTGGAAGGATGCTTCTGACCATCCC

a G S N S I Y P W S E V Q T F L R R L V G -  
AAACTGTTTATTGACCCAGAACAGATACAGGTGGGACTGGTACAGTATGGGGAGAGCCCT  
601 -----+-----+-----+-----+-----+ 660  
TTTGACAAATAACTGGGTCTTGTCTATGTCCACCCTGACCATGTCATACCCCTCTCGGGA

a K L F I D P E Q I Q V G L V Q Y G E S P -  
GTACATGAGTGGTCCCTGGGAGATTTCCGAACGAAGGAAGAAGTGGTGAGAGCAGCAAAG  
661 -----+-----+-----+-----+-----+ 720  
CATGTACTCACCAGGGACCCTCTAAAGGCTTGCTTCCTTCTTACCCTCTCGTCGTTTC

a V H E W S L G D F R T K E E V V R A A K -  
AACCTCAGTCGGCGGGAGGGACGAGAAACAAAGACTGCCCAAGCAATAATGGTGGCCTGC  
721 -----+-----+-----+-----+-----+ 780  
TTGGAGTCAGCCGCCCTCCCTGCTCTTTGTTTCTGACGGGTTCGTTATTACCACCGGACG

a N L S R R E G R E T K T A Q A I M V A C -  
ACAGAAGGGTTCAGTCAGTCCCATGGGGGCCGACCCGAGGCTGCCAGGCTACTGGTGGTT  
781 -----+-----+-----+-----+-----+ 840  
TGTCTTCCCAAGTCAGTCAGGGTACCCCGGCTGGGCTCCGACGGTCCGATGACCACCAA

a T E G F S Q S H G G R P E A A R L L V V -  
GTCACTGATGGAGAGTCCCATGATGGAGAGGAGCTTCCTGCAGCACTAAAGGCCTGTGAG  
841 -----+-----+-----+-----+-----+ 900  
CAGTGACTACCTCTCAGGGTACTACCTCTCCTCGAAGGACGTCGTGATTTCCGGACACTC

a V T D G E S H D G E E L P A A L K A C E -  
GCTGGAAGAGTGACACGCTATGGGATTGCAGTCCCTTGGTCACTACCTCCGGCGGCAGCGA  
901 -----+-----+-----+-----+-----+ 960  
CGACCTTCTCACTGTGCGATACCCTAACGTCAGGAACCACTGATGGAGGCCGCCGCTCGCT

a A G R V T R Y G I A V L G H Y L R R Q R -  
GATCCCAGCTCTTCTGAGAGAAATTAGAACTATTGCCAGTGATCCAGATGAGCGATTCT  
961 -----+-----+-----+-----+-----+ 1020  
CTAGGGTCGAGAAAGGACTCTCTTAATCTTGATAACGGTCACTAGGTCTACTCGCTAAG

a D P S S F L R E I R T I A S D P D E R F -  
TTCTTCAATGTACAGATGAGGCTGCTCTGACTGACATTGTGGATGCACTAGGAGATCGG  
1021 -----+-----+-----+-----+-----+ 1080  
AAGAAGTTACAGTGTCTACTCCGACGAGACTGACTGTAACACCTACGTGATCCTCTAGCC

a F F N V T D E A A L T D I V D A L G D R -  
ATTTTGGCCTTGAAGGGTCCCATGCAGAAAACGAAAGCTCCTTTGGGCTGGAAATGTCT  
1081 -----+-----+-----+-----+-----+ 1140  
TAAAAACCGGAACCTCCAGGGTACGTCTTTTGCTTTCGAGGAAACCCGACCTTTACAGA

45

a I F G L E G S H A E N E S S F G L E M S -  
CAGATTGGTTTCTCCACTCATCGGCTAAAGGATGGGATTCTTTTGGGATGGTGGGGGCC  
1141 -----+-----+-----+-----+-----+-----+ 1200  
GTCTAACCAGAGGTGAGTAGCCGATTTCCTACCCTAAGAAAAACCCTACCACCCCGG

a Q I G F S T H R L K D G I L F G M V G A -  
TATGACTGGGAGGCTCTGTGCTATGGCTTGAAGGAGGCCACCGCCTTTTCCCCCACGA  
1201 -----+-----+-----+-----+-----+-----+ 1260  
ATACTGACCCCTCCGAGACAGATACCGAACTTCCTCCGGTGGCGGAAAAGGGGGTGCT

a Y D W G G S V L W L E G G H R L F P P R -  
ATGGCACTGGAAGACGAGTTCCCCCTGCACTGCAGAACCATGCAGCCTACCTGGGTAC  
1261 -----+-----+-----+-----+-----+-----+ 1320  
TACCGTGACCTTCTGCTCAAGGGGGGACGTGACGTCTTGGTACGTGCGATGGACCCAATG

a M A L E D E F P P A L Q N H A A Y L G Y -  
TCTGTTTCTTCCATGCTTTTGC GG GTGGACGCCCTGTTTCTCTCTGGGGCTCCTCGA  
1321 -----+-----+-----+-----+-----+-----+ 1380  
AGACAAAGAAGGTACGAAACGCCCACTGCGGCGGACAAAGAGAGACCCCGAGGAGCT

a S V S S M L L R G G R R L F L S G A P R -  
TTTAGACATCGAGGAAAAGTCATCGCCTTCCAGCTTAAGAAAGATGGGGCTGTGAGGGTT  
1381 -----+-----+-----+-----+-----+-----+ 1440  
AAATCTGTAGCTCCTTTTCAGTAGCGGAAGGTGCAATTCTTTCTACCCCGACACTCCCAA

a F R H R G K V I A F Q L K K D G A V R V -  
GCCCAGAGCCTCCAGGGGAGCAGATTGGTTTCATACTTTGGCAGTGAGCTCTGCCCATTG  
1441 -----+-----+-----+-----+-----+-----+ 1500  
CGGGTCTCGGAGGTCCCCCTCGTCTAACCAAGTATGAAACCGTCACTCGAGACGGGTAAC

a A Q S L Q G E Q I G S Y F G S E L C P L -  
GATACAGATAGGGATGGAACAACCTGATGTCTTACTTGTGGCTGCCCCATGTTCTGGGA  
1501 -----+-----+-----+-----+-----+-----+ 1560  
CTATGTCTATCCCTACCTTGTGACTACAGATGAACACCGACGGGGGTACAAGGACCT

a D T D R D G T T D V L L V A A P M F L G -  
CCCCAGAACAAGGAAACAGGACGTGTTTATGTGTATCTGGTAGGCCAGCAGTCCTTGCTG  
1561 -----+-----+-----+-----+-----+-----+ 1620  
GGGGTCTTGTTCCTTTGTCTGCACAAATACACATAGACCATCCGGTCTGAGGAACGAC

a P Q N K E T G R V Y V Y L V G Q Q S L L -  
ACCCCTCCAAGGAACACTTCAGCCAGAACCCCCCAGGATGCTCGGTTTGGCTTTGCCATG  
1621 -----+-----+-----+-----+-----+-----+ 1680  
TGGGAGGTTCTTGTGAAGTCGGTCTTGGGGGGTCTACGAGCCAAACCGAAACGGTAC

a T L Q G T L Q P E P P Q D A R F G F A M -  
GGAGCTCTTCCTGATCTGAACCAAGATGGTTTTGCTGATGTGGCTGTGGGGGCGCCTCTG  
1681 -----+-----+-----+-----+-----+-----+ 1740  
CCTCGAGAAGGACTAGACTTGGTTCTACCAAAACGACTACACCGACACCCCCGCGGAGAC

a G A L P D L N Q D G F A D V A V G A P L -  
GAAGATGGGCACCAGGGAGCACTGTACCTGTACCATGGAACCCAGAGTGGAGTCAGGCCC  
1741 -----+-----+-----+-----+-----+-----+ 1800  
CTTCTACCCGTGGTCCCTCGTGACATGGACATGGTACCTTGGGTCTACCTCAGTCCGGG



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a E D G H Q G A L Y L Y H G T Q S G V R P -  
CATCCTGCCCAGAGGATTGCTGCTGCCTCCATGCCACATGCCCTCAGCTACTTTGGCCGA  
1801 -----+-----+-----+-----+-----+ 1860  
GTAGGACGGGTCTCCTAACGACGACGGAGGTACGGTGTACGGGAGTCGATGAAACCGGCT

a H P A Q R I A A A S M P H A L S Y F G R -  
AGTGTGGATGGTCGGCTAGATCTGGATGGAGATGATCTGGTCGATGTGGCTGTGGGTGCC  
1861 -----+-----+-----+-----+-----+ 1920  
TCACACCTACCAGCCGATCTAGACCTACCTCTACTAGACCAGCTACACCGACACCCACGG

a S V D G R L D L D G D D L V D V A V G A -  
CAGGGGGCAGCCATCCTGCTCAGCTCCCGGCCATTGTCCATCTGACCCCATCACTGGAG  
1921 -----+-----+-----+-----+-----+ 1980  
GTCCCCCGTCGGTAGGACGAGTCGAGGGCCGGTAACAGGTAGACTGGGGTAGTGACCTC

a Q G A A I L L S S R P I V H L T P S L E -  
GTGACCCACAGGCCATCAGTGTGGTTCAGAGGGACTGTAGGCGGCGAGGCCAAGAAGCA  
1981 -----+-----+-----+-----+-----+ 2040  
CACTGGGGTGTCCGGTAGTCACACCAAGTCTCCCTGACATCCGCCGCTCCGGTTCTTCGT

a V T P Q A I S V V Q R D C R R R G Q E A -  
GTCTGTCTGACTGCAGCCCTTTGCTTCCAAGTGACCTCCCGTACTCCTGGTCGCTGGGAT  
2041 -----+-----+-----+-----+-----+ 2100  
CAGACAGACTGACGTCGGGAAACGAAGGTTCACTGGAGGGCATGAGGACCAGCGACCCTA

a V C L T A A L C F Q V T S R T P G R W D -  
CACCAATTCTACATGAGGTTCAACCGCATCACTGGATGAATGGACTGCTGGGGCACGTGCA  
2101 -----+-----+-----+-----+-----+ 2160  
GTGGTTAAGATGTACTCCAAGTGCGTAGTGACCTACTTACCTGACGACCCCGTGACGT

a H Q F Y M R F T A S L D E W T A G A R A -  
GCATTTGATGGCTCTGGCCAGAGTTGTCCCTCGGAGGCTCCGGCTCAGTGTGGGGAAT  
2161 -----+-----+-----+-----+-----+ 2220  
CGTAAACTACCAGACCGGTCTCCAACAGGGGAGCCTCCGAGGCCGAGTCACACCCCTTA

a A F D G S G Q R L S P R R L R L S V G N -  
GTCACCTTGTGAGCAGCTACACTTCCATGTGCTGGATACATCAGATTACCTCCGGCCAGTG  
2221 -----+-----+-----+-----+-----+ 2280  
CAGTGAACACTCGTCGATGTGAAGGTACACGACCTATGTAGTCTAATGGAGGCCGGTCAC

a V T C E Q L H F H V L D T S D Y L R P V -  
GCCTTGACTGTGACCTTTGCCTTGGACAATACTACAAAGCCAGGGCCTGTGCTGAATGAG  
2281 -----+-----+-----+-----+-----+ 2340  
CGGAACCTGACACTGGAAACGGAACCTGTTATGATGTTTCGGTCCCGGACACGACTTACTC

a A L T V T F A L D N T T K P G P V L N E -  
GGCTCACCACCTCTATACAAAGCTGGTCCCTTCTCAAAGGATTGTGGCCCTGACAAT  
2341 -----+-----+-----+-----+-----+ 2400  
CCGAGTGGGTGGAGATATGTTTTGACCAGGGGAAGAGTTTCCTAACACCGGGACTGTTA

a G S P T S I Q K L V P F S K D C G P D N -  
GAATGTGTACAGACCTGGTGCTTCAAGTGAATATGGACATCAGAGGCTCCAGGAAGGCC  
2401 -----+-----+-----+-----+-----+ 2460  
CTTACACAGTGTCTGGACCACGAAGTTCATTATACCTGTAGTCTCCGAGGTCCTTCCGG

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a E C V T D L V L Q V N M D I R G S R K A -  
CCATTGTGTTTCGAGGTGGCCGGCGAAAGTGCTGGTATCTACAACCTCTGGAGAACAGA  
2461 -----+-----+-----+-----+-----+-----+ 2520  
GGTAAACACCAAGCTCCACCGGCCGCTTTACGACCATAGATGTTGAGACCTCTTGCTCT

a P F V V R G G R R K V L V S T T L E N R -  
AAGGAAAATGCTTACAATACGAGCCTGAGTATCATCTTCTCTAGAAACCTCCACCTGGCC  
2521 -----+-----+-----+-----+-----+-----+ 2580  
TTCCTTTTACGAATGTTATGCTCGGACTCATAGTAGAAGAGATCTTTGGAGGTGGACCGG

a K E N A Y N T S L S I I F S R N L H L A -  
AGTCTCACTCCTCAGAGAGAGAGCCCAATAAAGGTGGAATGTGCCGCCCTTCTGCTCAT  
2581 -----+-----+-----+-----+-----+-----+ 2640  
TCAGAGTGAGGAGTCTCTCTCTCGGGTTATTTCCACCTTACACGGCGGGAAGACGAGTA

a S L T P Q R E S P I K V E C A A P S A H -  
GCCCCGCTCTGCAGTGTGGGGCATCCTGTCTTCCAGACTGGAGCCAAGGTGACCTTTCTG  
2641 -----+-----+-----+-----+-----+-----+ 2700  
CGGGCCGAGACGTACACCCCGTAGGACAGAAGGTCTGACCTCGGTTCCACTGGAAGAC

a A R L C S V G H P V F Q T G A K V T F L -  
CTAGAGTTTGAGTTAGCTGCTCCTCTCTCTGAGCCAGGTCTTTGGGAAGCTGACTGCC  
2701 -----+-----+-----+-----+-----+-----+ 2760  
GATCTCAAACCTCAAATCGACGAGGAGAGAGGACTCGGTCCAGAAACCCTTCGACTGACGG

a L E F E F S C S S L L S Q V F G K L T A -  
AGCAGTGACAGCCTGGAGAGAAATGGCACCCTTCAAGAAAACACAGCCCAGACCTCAGCC  
2761 -----+-----+-----+-----+-----+-----+ 2820  
TCGTCACTGTCCGACCTCTCTTTACCGTGGGAAGTTCTTTTGTGTCCGGTCTGGAGTCGG

a S S D S L E R N G T L Q E N T A Q T S A -  
TACATCCAATATGAGCCCCACCTCCTGTTCTCTAGTGAGTCTACCCTGCACCGCTATGAG  
2821 -----+-----+-----+-----+-----+-----+ 2880  
ATGTAGGTTATACTCGGGGTGGAGGACAAGAGATCACTCAGATGGGACGTGGCGATACTC

a Y I Q Y E P H L L F S S E S T L H R Y E -  
GTTCACCCATATGGGACCCTCCCAGTGGGTCTGGCCCAGAATTCAAACCACTCTCAGG  
2881 -----+-----+-----+-----+-----+-----+ 2940  
CAAGTGGGTATACCCTGGGAGGGTCACCCAGGACCGGGTCTTAAGTTTGGTGAGAGTCC

a V H P Y G T L P V G P G P E F K T T L R -  
ACTAACAATGCAAGCTGCATAGTGCAGAACCTGACTGAACCCCCAGGCCACCTGTGCAT  
2941 -----+-----+-----+-----+-----+-----+ 3000  
TGATTGTTACGTTTCGACGTATCACGTCTTGGACTGACTTGGGGTCCGGGTGGACACGTA

a T N N A S C I V Q N L T E P P G P P V H -  
CCAGAGGAGCTTCAACACACAAACAGACTGAATGGGAGCAATACTCAGTGTGAGGTGGTG  
3001 -----+-----+-----+-----+-----+-----+ 3060  
GGTCTCCTCGAAGTTGTGTGTTGTCTGACTTACCCTCGTTATGAGTCACAGTCCACCAC

a P E E L Q H T N R L N G S N T Q C Q V V -  
AGGTGCCACCTTGGGCAGCTGGCAAAGGGGACTGAGGTCTCTGTTGGACTATTGAGGCTG  
3061 -----+-----+-----+-----+-----+-----+ 3120  
TCCACGGTGAACCCGTCGACCGTTTCCCTGACTCCAGAGACAACCTGATAACTCCGAC

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a R C H L G Q L A K G T E V S V G L L R L -  
GTT CACAATGAATTTTCCGAAGAGCCAAGTTCAGTCCCTGACGGTGGTCAGCACCTTT  
3121 -----+-----+-----+-----+-----+ 3180  
CAAGTGTTACTTAAAAAGGCTTCTCGGTTCAAGTTCAGGGACTGCCACCAGTCGTGGAAA

a V H N E F F R R A K F K S L T V V S T F -  
GAGCTGGGAACCGAAGAGGGCAGTGTCTACAGCTGACTGAAGCCTCCCGTTGGAGTGAG  
3181 -----+-----+-----+-----+-----+ 3240  
CTCGACCCTTGGCTTCTCCCGTCACAGGATGTGACTGACTTCGGAGGGCAACCTCACTC

a E L G T E E G S V L Q L T E A S R W S E -  
AGCCTCTTGGAGGTGGTTCAGACCCGGCCTATCTCATCTCCCTGTGGATCCTCATAGGC  
3241 -----+-----+-----+-----+-----+ 3300  
TCGGAGAACCTCCACCAAGTCTGGCCGGATAGGAGTAGAGGGACACCTAGGAGTATCCG

a S L L E V V Q T R P I L I S L W I L I G -  
AGTGTCTTGGGAGGGTTGCTCCTGCTTGTCTCTTGTCTTCTGCTGTGGAAGCTTGGC  
3301 -----+-----+-----+-----+-----+ 3360  
TCACAGGACCTCCCAACGAGGACGAACGAGAGGAACAGAAGACGGACACCTTCGAACCG

a S V L G G L L L L A L L V F C L W K L G -  
TTCTTTGCCATAAGAAAATCCCTGAGGAAGAAAAAGAGAAGAGAAGTTGGAGCAATGA  
3361 -----+-----+-----+-----+-----+ 3420  
AAGAAACGGGTATTCTTTTAGGGACTCCTTCTTTTCTCTTCTTCAACCTCGTTACT

a F F A H K K I P E E E K R E E K L E Q  
ATGTAGAATAAGGGTCTAGAAAGTCCCTCCCTGGCAGCTTTCTTCAAGAGACTTGCATAAA  
3421 -----+-----+-----+-----+-----+ 3480  
TACATCTTATTCCCAGATCTTTTCAGGAGGGACCGTCGAAAGAAGTTCTCTGAACGTATTT

AGCAGAGGTTTGGGGGCTCAGATGGGACAAGAAGCCGCCTCTGGACTATCTCCCCAGACC  
3481 -----+-----+-----+-----+-----+ 3540  
TCGTCTCCAAACCCCCGAGTCTACCCTGTTCTTCGGCGGAGACCTGATAGAGGGGTCTGG

AGCAGCCTGACTTGACTTTTGAGTCCTAGGGATGCTGCTGGCTAGAGATGAGGCTTTACC  
3541 -----+-----+-----+-----+-----+ 3600  
TCGTCTGACTGAACTGAAAACCTCAGGATCCCTACGACGACCGATCTCTACTCCGAAATGG

TCAGACAAGAAGAGCTGGCACCAAAACTAGCCATGCTCCACCCCTCTGCTTCCCTCCTCC  
3601 -----+-----+-----+-----+-----+ 3660  
AGTCTGTTCTTCTCGACCGTGGTTTGTGATCGGTACGAGGGTGGGAGACGAAGGGAGGAGG

TCGTGATCCTGGTTCCATAGCCAACACTGGGGCTTTTGTGTTGGGGTCTTTTATCCCCAG  
3661 -----+-----+-----+-----+-----+ 3720  
AGCACTAGGACCAAGGTATCGGTTGTGACCCGAAAAACAAACCCAGGAAAATAGGGGTC

GAATCAATAATTTTTTGCCTAGGAAAAAAAAGCGGCCGGAATTCGATATCAAGCT  
3721 -----+-----+-----+-----+-----+ 3779  
CTTAGTTATTAAAAAACGGATCCTTTTTTTTTTCGCCGGCGCTTAAGCTATAGTTCTGA

## (2) INFORMATION FOR SEQ ID NO. 3:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 143 base pairs  
    (B) TYPE: nucleic acid and amino acid  
    (C) STRANDEDNESS: double  
    (D) TOPOLOGY: linear
- (iii) MOLECULAR TYPE: cDNA
- (vi) ORIGINAL SOURCE:  
    (A) ORGANISM: human  
    (B) CELLTYPE: chondrocyte
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO. 3:

NdeI

1 GGGGCATATGGTTTCAGAACCTGGGTTGCTACGTTGTTTCCGGTCTGATCATCTCCGCTCT  
-----+-----+-----+-----+-----+ 60  
CCCCGTATACCAAGTCTTGGACCCAACGATGCAACAAAGGCCAGACTAGTAGAGGCGAGA

b       G H M V Q N L G C Y V V S G L I I S A L -

61 GCTGCCGGCTGTTGCTCACGGTGGTAACTACTTCCTAAGCTTGTCCCAGGTTATCAGCGG  
-----+-----+-----+-----+-----+ 120  
CGACGGCCGACAACGAGTGCCACCATTGATGAAGGATTCGAACAGGGTCCAATAGTCGCC

b       L P A V A H G G N Y F L S L S Q V I S G -

BamHI

121 CCTGGTGCCGCGCGGATCCCCC  
-----+-----+----- 143  
GGACCACGGCGCGCCTAGGGGGG

b       L V P R G S P -

CLAIMS

1. A recombinant or isolated collagen binding  
integrin subunit  $\alpha 10$  comprising essentially the amino  
acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or  
homologues or fragments thereof having essentially the  
same biological activity.

2. A process of producing a recombinant integrin  
subunit  $\alpha 10$  comprising essentially the amino acid  
sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or  
homologues or fragments thereof having essentially the  
same biological activity, which process comprises the  
steps of

a) isolating a polynucleotide comprising a nucleo-  
tide sequence coding for an integrin subunit  $\alpha 10$ , or  
homologues or fragments thereof having essentially the  
same biological activity,

b) constructing an expression vector comprising the  
isolated polynucleotide,

c) transforming a host cell with said expression  
vector,

d) culturing said transformed host cell in a culture  
medium under conditions suitable for expression of inte-  
grin subunit  $\alpha 10$ , or homologues or fragments thereof hav-  
ing essentially the same biological activity, in said  
transformed host cell, and, optionally,

e) isolating the integrin subunit  $\alpha 10$ , or homologues  
or fragments thereof having essentially the same  
biological activity, from said transformed host cell or  
said culture medium.

3. A process of providing an integrin subunit  $\alpha 10$ ,  
or homologues or fragments thereof having essentially the  
same biological activity, whereby said subunit is  
isolated from a cell in which it is naturally present.

4. An isolated polynucleotide comprising a nucleo-  
tide coding for an integrin subunit  $\alpha 10$ , or for homolo-  
gues or fragments thereof having essentially the same

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biological activity, which polynucleotide comprises essentially the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2 or suitable parts thereof.

5 5. An isolated polynucleotide or oligonucleotide which hybridises to a DNA or RNA coding for an integrin subunit  $\alpha 10$ , or for homologues or fragments thereof having essentially the same biological activity, where-  
10 in said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit  $\alpha 1$ .

15 6. A vector comprising a polynucleotide or oligonucleotide coding for an integrin subunit  $\alpha 10$ , or for homologues or fragments thereof having essentially the same biological activity, which polynucleotide or oligonucleotide comprises essentially the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2 or parts thereof.

20 7. A vector comprising a polynucleotide or oligonucleotide which hybridises to a DNA or RNA coding for an integrin subunit  $\alpha 10$ , or for homologues or fragments thereof, wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit  $\alpha 1$ .

25 8. A cell containing the vector as defined in any one of claims 6 and 7.

30 9. A cell generated by steps a) to d) of the process as defined in claim 2, in which a polynucleotide or oligonucleotide coding for an integrin subunit  $\alpha 10$ , or for homologues or fragments thereof having essentially the same biological activity, which polynucleotide or oligonucleotide comprises the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2 or parts thereof, has been stably integrated in the cell genome.

35 10. Binding entities having the capability of binding specifically to an integrin subunit  $\alpha 10$  comprising the amino acid sequence of SEQ ID No. 1 or SEQ ID No. 2, or to homologues or fragments thereof.

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11. Binding entities according to claim 10, which are chosen from the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, and fragments thereof.

5 12. Binding entities according to claim 10, which are polyclonal or monoclonal antibodies, or fragments thereof.

13. A recombinant or isolated integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , in which the  
10 subunit  $\alpha 10$  comprises essentially the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, and homologues and fragments thereof having essentially the same biological activity.

14. A recombinant or isolated integrin heterodimer  
15 according to claim 13, wherein the subunit  $\beta$  is  $\beta 1$ .

15. A process of producing a recombinant integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , in which the subunit  $\alpha 10$  comprises essentially the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, and  
20 homologues and fragments thereof having essentially the same biological activity, which process comprises the steps of

a) isolating one polynucleotide comprising a nucleotide sequence coding for a subunit  $\alpha 10$  of an integrin  
25 heterodimer and, optionally, another polynucleotide comprising a nucleotide sequence coding for a subunit  $\beta$  of an integrin heterodimer, or polynucleotides or oligonucleotides coding for homologues or fragments thereof having essentially the same biological activity,

30 b) constructing an expression vector comprising said isolated polynucleotide coding for said subunit  $\alpha 10$  optionally in combination with an expression vector comprising said isolated nucleotide coding for said subunit  $\beta$ ,

35 c) transforming a host cell with said expression vector or vectors,

d) culturing said transformed host cell in a culture medium under conditions suitable for expression of an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or homologues or fragments thereof having essentially the same biological activity, in said transformed host cell, and, optionally,

e) isolating the integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or homologues or fragments thereof having essentially the same biological activity, or the  $\alpha 10$  subunit thereof from said transformed host cell or said culture medium.

16. A process of providing a integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or homologues or fragments thereof having essentially the same biological activity, whereby said integrin heterodimer is isolated from a cell in which it is naturally present.

17. A cell containing a first vector, said first vector comprising a polynucleotide or oligonucleotide coding for a subunit  $\alpha 10$  of an integrin heterodimer, or for homologues or parts thereof having essentially the same biological activity, which polynucleotide or oligonucleotide comprises essentially the nucleotide sequence shown in SEQ ID No. 1 or SEQ ID No. 2 or parts thereof, and a second vector, said second vector comprising a polynucleotide or oligonucleotide coding for a subunit  $\beta$  of an integrin heterodimer, or for homologues or fragments thereof having essentially the same biological activity.

18. Binding entities having the capability of binding specifically to an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having essentially the same biological activity, or an subunit  $\alpha 10$  thereof, having essentially the same biological activity.

19. Binding entities according to claim 18, wherein the subunit  $\beta$  is  $\beta 1$ .



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20. Binding entities according to claim 18 or 19, which are chosen among the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, and fragments thereof.

5 21. Binding entities according to claim 18 or 19, which are polyclonal or monoclonal antibodies

22. A fragment of the integrin subunit  $\alpha 10$ , which fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the  
10 spliced domain.

23. A fragment according to claim 22, which is a peptide comprising the amino acid sequence  
KLGFFAHKKIPEEEKREEKLEQ.

24. A fragment according to claim 22, which com-  
15 prises the amino acid sequence from about amino acid No. 952 to about amino acid no. 986 of SEQ ID No. 1.

25. A fragment according to claim 22, which is a peptide comprising the amino acid sequence from about amino acid No. 140 to about amino acid no. 337 of  
20 SEQ ID No. 1.

26. A method of producing a fragment of the integrin subunit  $\alpha 10$  as defined in any one of claims 22-25, which method comprises a sequential addition of amino acids containing protective groups.

25 27. A polynucleotide or oligonucleotide coding for a fragment of the integrin subunit  $\alpha 10$  as defined in any one of claims 22-25.

28. Binding entities having the capability of binding specifically to a fragment of the human integrin sub-  
30 unit  $\alpha 10$  as defined in any one of claims 22-25.

29. Binding entities according to claim 28, which are chosen from the group comprising proteins, peptides, carbohydrates, lipids, natural integrin binding ligands, and fragments thereof.

35 30. Binding entities according to claim 28, which are polyclonal or monoclonal antibodies, or fragments thereof.

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31. An *in vitro* process of using an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or a  
5 homologue or fragment of said integrin or subunit having essentially the same biologically activity, as a marker or target molecule of cells or tissues expressing said integrin subunit  $\alpha 10$ , which cells or tissues are of animal including human origin.

10 32. An *in vitro* process according to claim 31, whereby said fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

15 33. An *in vitro* process according to claim 31, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

20 34. An *in vitro* process according to claim 31, whereby said fragment comprises the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

35 35. An *in vitro* process according to claim 31, whereby said fragment comprises the amino acid sequence from about amino acid no. 140 to about amino acid no. 337 of SEQ ID No. 1.

25 36. An *in vitro* process according to claim 31, whereby the subunit  $\beta$  is  $\beta 1$ .

30 37. An *in vitro* process according to claim 31, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

38. An *in vitro* process according to any one of claims 31-37, which process is used during pathological conditions involving said subunit  $\alpha 10$ .

35 39. An *in vitro* process according to claim 38, which pathological conditions comprise damage of cartilage.

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40. An *in vitro* process according to claim 38, which pathological conditions comprise trauma, rheumatoid arthritis and osteoarthritis.

41. An *in vitro* process according to any one of  
5 claims 31-37, which is a process for detecting the formation of cartilage during embryonal development.

42. An *in vitro* process according to any one of claims 31-37, which is a process for detecting physiological or therapeutic reparation of cartilage.

10 43. An *in vitro* process according to any one of claims 31-37, which is a process for selection and analysis, or for sorting, isolating or purification of chondrocytes.

44. An *in vitro* process according to any one of  
15 claims 31-37, which is a process for detecting regeneration of cartilage or chondrocytes during transplantation of cartilage or chondrocytes.

45. A process according to any one of claims 31-37, which is a process for *in vitro* studies of differentia-  
20 tion of chondrocytes.

46. An *in vitro* process of using binding entities having the capability of binding specifically to an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin  
25 heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having essentially the same biological activity, as markers or target molecules of cells or tissues expressing said integrin subunit  $\alpha 10$ , which cells or tissues are of animal  
30 including human origin.

47. An *in vitro* process according to claim 46, whereby said fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

35 48. An *in vitro* process according to claim 46, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

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49. An *in vitro* process according to claim 46,  
whereby said fragment comprises the amino acid sequence  
from about amino acid no. 952 to about amino acid no. 986  
of SEQ ID No. 1.

5 50. An *in vitro* process according to claim 46,  
whereby said fragment comprises the amino acid sequence  
from about amino acid no. 140 to about amino acid No. 337  
of SEQ ID No. 1.

10 51. An *in vitro* process according to claim 46,  
whereby the subunit  $\beta$  is  $\beta 1$ .

52. An *in vitro* process according to any one of  
claims 46-51, which is a process for detecting the  
presence of an integrin subunit  $\alpha 10$  comprising the amino  
acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or  
15 of an integrin heterodimer comprising said subunit  $\alpha 10$   
and a subunit  $\beta$ , or of homologues or fragments thereof  
having essentially the same biological activity.

53. An *in vitro* process according to any one of  
claims 46-51, which process is a process for determining  
20 the differentiation-state of cells during embryonic  
development, angiogenesis, or development of cancer.

54. An *in vitro* process for detecting the presence  
of a integrin subunit  $\alpha 10$ , or of a homologue or fragment  
of said integrin subunit having essentially the same  
25 biological activity, on cells, whereby a polynucleotide  
or oligonucleotide chosen from the group comprising a  
polynucleotide or oligonucleotide shown in SEQ ID No. 1  
is used as a marker under hybridisation conditions  
wherein said polynucleotide or oligonucleotide fails to  
30 hybridise to a DNA or RNA encoding an integrin subunit  
 $\alpha 1$ .

55. An *in vitro* process according to claim 54,  
whereby said cells are chosen from the group comprising  
chondrocytes, smooth muscle cells, endothelial cells,  
35 osteoblasts and fibroblasts.

56. An *in vitro* process according to claim 54,  
whereby said fragment is a peptide chosen from the group

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comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

57. An *in vitro* process according to claim 54, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

58. An *in vitro* process according to claim 54, whereby said fragment comprises the amino acid sequence from about amino acid No. 952 to about amino acid no. 986 of SEQ ID No. 1.

59. An *in vitro* process according to claim 54, whereby said fragment comprises the amino acid sequence from about amino acid No. 140 to about amino acid No. 337 of SEQ ID No. 1.

60. An *in vitro* process according to any one of claims 54-59, which is a process for determining the differentiation-state of cells during development, in pathological conditions, in tissue regeneration or in therapeutic and physiological reparation of cartilage.

61. An *in vitro* process according to claim 60, wherein the pathological conditions are any pathological conditions involving the integrin subunit  $\alpha 10$ .

62. An *in vitro* process according to claim 61, whereby said pathological conditions are rheumatoid arthritis, osteoarthritis or cancer.

63. An *in vitro* process according to claim 60, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

64. An *in vitro* process for determining the differentiation-state of cells during development, in pathological conditions, in tissue regeneration and in therapeutic and physiological reparation of cartilage, whereby a polynucleotide or oligonucleotide chosen from the nucleotide sequence shown in SEQ ID No. 1 is used as a marker under hybridisation conditions wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit  $\alpha 1$ .

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65. An *in vitro* process according to claim 64,  
whereby said polynucleotide or oligonucleotide is a  
polynucleotide or oligonucleotide coding for a peptide  
chosen from the group comprising peptides of the  
5 cytoplasmic domain, the I-domain and the spliced domain.

66. An *in vitro* process according to claim 65,  
whereby said polynucleotide or oligonucleotide is a  
polynucleotide or oligonucleotide coding for a peptide  
comprising the amino acid sequence  
10 KLGFFAHKKIPEEEEKREEKLEQ.

67. An *in vitro* process according to claim 65,  
whereby said peptide comprises the amino acid sequence  
from about amino acid no. 952 to about amino acid no. 986  
of SEQ ID No. 1.

68. An *in vitro* process according to claim 65,  
whereby said peptide comprises the amino acid sequence  
from about amino acid no. 140 to about amino acid no. 337  
of SEQ ID No. 1.

69. An *in vitro* process according to claim 65,  
20 whereby said pathological conditions are any pathological  
conditions involving the integrin subunit  $\alpha 10$ .

70. An *in vitro* process according to claim 69,  
whereby said pathological conditions are rheumatoid  
arthritis, osteoarthritis or cancer.

71. An *in vitro* process according to claim 69,  
25 whereby said pathological conditions are atherosclerosis  
or inflammation.

72. An *in vitro* process according to any one of  
claims 64-71, whereby said cells are chosen from the  
30 group comprising chondrocytes, smooth muscle cells,  
endothelial cells, osteoblasts and fibroblasts.

73. A pharmaceutical composition comprising as an  
active ingredient a pharmaceutical agent or an antibody  
which is capable of using an integrin heterodimer com-  
35 prising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$   
thereof, or a homologue or fragment of said integrin or

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subunit  $\alpha 10$  having essentially the same biological activity, as a target molecule.

74. A pharmaceutical composition according to claim 73, for use in stimulating, inhibiting or blocking the formation of cartilage, bone or blood vessels.

75. A pharmaceutical composition according to claim 73, for use in preventing adhesion between tendon/ligaments and the surrounding tissue after infection, inflammation and after surgical intervention where adhesion impairs the function of the tissue.

76. A vaccine comprising as an active ingredient an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit  $\alpha 10$ , or DNA or RNA coding for said integrin subunit  $\alpha 10$ .

77. *In vitro* use of the integrin subunit  $\alpha 10$  as a marker or target in transplantation of cartilage or chondrocytes.

78. An *in vitro* method of using binding entities having the capability of binding specifically to an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having essentially the same biological activity, for promoting adhesion of chondrocytes and/or osteoblasts to surfaces of implants to stimulate osseointegration.

79. A method of *in vitro* detecting the presence of integrin binding entities, comprising interaction of an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit having essentially the same biological activity, with a sample, thereby causing said integrin, subunit  $\alpha 10$ , or homologue or fragment thereof, to modulate the binding to its natural ligand or other integrin binding proteins present in said sample.

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80. A method of *in vitro* studying consequences of the interaction of a human heterodimer integrin comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit having essentially the same biological activity, with an integrin binding entity and thereby initiate a cellular reaction.

81. A method according to claim 80, whereby the consequences of said interactions are measured as alterations in cellular functions.

82. An *in vitro* method of using DNA or RNA encoding an integrin subunit  $\alpha 10$  or homologues or fragments thereof as a target molecule.

83. An *in vitro* method according to claim 82, whereby a polynucleotide or oligonucleotide hybridises to the DNA or RNA encoding an integrin subunit  $\alpha 10$ , or homologues or fragments thereof having essentially the same biological activity, and whereby said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit  $\alpha 1$ .

84. An *in vitro* method of using a human heterodimer integrin comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit, or a DNA or RNA encoding an integrin subunit  $\alpha 10$  or homologues or fragments thereof, as a marker or target molecule during angiogenesis.

85. A pharmaceutical composition comprising as an active ingredient a pharmaceutical agent or an antibody which is capable of stimulating cell surface expression of an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit  $\alpha 10$  having essentially the same biological activity.

86. A process of using a collagen binding integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or a

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homologue or fragment of said integrin or subunit having essentially the same biologically activity, as a marker or target molecule of cells or tissues expressing said integrin subunit  $\alpha 10$ , which cells or tissues are of animal including human origin.

87. A process according to claim 86, whereby said fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

88. A process according to claim 86, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

89. A process according to claim 86, whereby said fragment comprises the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

90. A process according to claim 86, whereby said fragment comprises the amino acid sequence from about amino acid no. 140 to about amino acid no. 337 of SEQ ID No. 1.

91. A process according to claim 86, whereby the subunit  $\beta$  is  $\beta 1$ .

92. A process according to claim 86, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

93. A process according to any one of claims 86-92, which process is used during pathological conditions involving said subunit  $\alpha 10$ .

94. A process according to claim 93, which pathological conditions comprise damage of cartilage.

95. A process according to claim 93, which pathological conditions comprise trauma, rheumatoid arthritis and osteoarthritis.

96. A process according to any one of claims 86-92, which is a process for detecting the formation of cartilage during embryonal development.

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97. A process according to any one of claims 86-92, which is a process for detecting physiological or therapeutic reparation of cartilage.

98. A process according to any one of claims 86-92, which is a process for detecting regeneration of cartilage or chondrocytes during transplantation of cartilage or chondrocytes.

99. A process of using binding entities having the capability of binding specifically to an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having essentially the same activity, as markers or target molecules of cells or tissues expressing said integrin subunit  $\alpha 10$ , which cells or tissues are of animal including human origin.

100. A process according to claim 99, whereby said fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

101. A process according to claim 99, whereby said fragment is a peptide comprising the amino acid sequence KLGFFAHKKIPEEEKREEKLEQ.

102. A process according to claim 99, whereby said fragment comprises the amino acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

103. A process according to claim 99, whereby said fragment comprises the amino acid sequence from about amino acid no. 140 to about amino acid No. 337 of SEQ ID No. 1.

104. A process according to claim 99, whereby the subunit  $\beta$  is  $\beta 1$ .

105. A process according to any one of claims 99-104, which is a process for detecting the presence of an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or of an integrin

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heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or of homologues or fragments thereof having essentially the same biologically activity.

106. A process according to any one of claims 99-  
5 104, which process is a process for determining the differentiation-state of cells during embryonic development, angiogenesis, or development of cancer.

107. A process for detecting the presence of an  
10 integrin subunit  $\alpha 10$ , or of a homologue or fragment of said integrin subunit having essentially the same activity, on cells, whereby a polynucleotide or oligonucleotide chosen from the group comprising a polynucleotide or oligonucleotide shown in SEQ ID No. 1 is used as a marker under hybridisation conditions  
15 wherein said polynucleotide or oligonucleotide fails to hybridise to a DNA or RNA encoding an integrin subunit  $\alpha 1$ .

108. A process according to claim 107, whereby said  
20 cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

109. A process according to claim 107, whereby said  
25 fragment is a peptide chosen from the group comprising peptides of the cytoplasmic domain, the I-domain and the spliced domain.

110. A process according to claim 107, whereby said  
fragment is a peptide comprising the amino acid sequence  
KLGFFAHKKIPEEEKREEKLEQ.

111. A process according to claim 107, whereby said  
30 fragment comprises the amino acid sequence from about amino acid No. 952 to about amino acid no. 986 of SEQ ID No. 1.

112. A process according to claim 107, whereby said  
35 fragment comprises the amino acid sequence from about amino acid No. 140 to about amino acid No. 337 of SEQ ID No. 1.

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113. A process according to any one of claims 107-  
112, which is a process for determining the  
differentiation-state of cells during development, in  
pathological conditions, in tissue regeneration or in  
5 therapeutic and physiological reparation of cartilage.

114. A process according to claim 113, wherein the  
pathological conditions are any pathological conditions  
involving the integrin subunit  $\alpha 10$ .

115. A process according to claim 113, whereby said  
10 pathological conditions are rheumatoid arthritis, osteo-  
arthrosis or cancer.

116. A process according to claim 113, whereby said  
cells are chosen from the group comprising chondrocytes,  
smooth muscle cells, endothelial cells, osteoblasts and  
15 fibroblasts.

117. A process for determining the differentiation-  
state of cells during development, in pathological con-  
ditions, in tissue regeneration and in therapeutic and  
physiological reparation of cartilage, whereby a poly-  
20 nucleotide or oligonucleotide chosen from the nucleotide  
sequence shown in SEQ ID No. 1 is used as a marker under  
hybridisation conditions wherein said polynucleotide or  
oligonucleotide fails to hybridise to a DNA or RNA encod-  
ing an integrin subunit  $\alpha 1$ .

25 118. A process according to claim 117, whereby said  
polynucleotide or oligonucleotide is a polynucleotide or  
oligonucleotide coding for a peptide chosen from the  
group comprising peptides of the cytoplasmic domain, the  
I-domain and the spliced domain.

30 119. A process according to claim 117, whereby said  
polynucleotide or oligonucleotide is a polynucleotide or  
oligonucleotide coding for a peptide comprising the amino  
acid sequence KLGFFAHKKIPEEEKREEKLEQ.

120. A process according to claim 117, whereby said  
35 polynucleotide or oligonucleotide is a polynucleotide or  
oligonucleotide coding for a peptide comprising the amino

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acid sequence from about amino acid no. 952 to about amino acid no. 986 of SEQ ID No. 1.

121. A process according to claim 117, whereby said polynucleotide or oligonucleotide is a polynucleotide or oligonucleotide coding for a peptide comprising the amino acid sequence from about amino acid no. 140 to about amino acid no. 337 of SEQ ID No. 1.

122. A process according to claim 117, whereby said pathological conditions are any pathological conditions involving the integrin subunit  $\alpha 10$ .

123. A process according to claim 117, whereby said pathological conditions are rheumatoid arthritis, osteoarthritis or cancer.

124. A process according to claim 117, whereby said pathological conditions are atherosclerosis or inflammation.

125. A process according to any one of claims 117-124, whereby said cells are chosen from the group comprising chondrocytes, smooth muscle cells, endothelial cells, osteoblasts and fibroblasts.

126. A method of using an integrin subunit  $\alpha 10$  as defined in claim 1 as a marker or target in transplantation of cartilage or chondrocytes.

127. A method of using binding entities having the capability of binding specifically to an integrin subunit  $\alpha 10$  comprising the amino acid sequence shown in SEQ ID No. 1 or SEQ ID No. 2, or an integrin heterodimer comprising said subunit  $\alpha 10$  and a subunit  $\beta$ , or to homologues or fragments thereof having essentially the same biological activity, for promoting adhesion of chondrocytes and/or osteoblasts to surfaces of implants to stimulate osseointegration.

128. Use of an integrin heterodimer comprising an integrin subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or fragment of said integrin or subunit  $\alpha 10$  having essentially the same biological activity, as a target for anti-adhesive drugs or

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molecules in tendon, ligament, skeletal muscle or other tissues where adhesion impairs the function of the tissue.

129. A method of stimulating, inhibiting or blocking  
5 the formation of cartilage or bone, comprising administration to a subject a suitable amount of a pharmaceutical agent or an antibody which is capable of using an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or  
10 fragment of said integrin or subunit  $\alpha 10$  having essentially the same biological activity, as a target molecule.

130. A method of preventing adhesion between tendon/  
ligaments and the surrounding tissue after infection,  
15 inflammation and after surgical intervention where adhesion impairs the function of the tissue, comprising administration to a subject a suitable amount of a pharmaceutical agent or an antibody which is capable of using an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  $\alpha 10$  thereof, or a homologue or  
20 fragment of said integrin or subunit  $\alpha 10$  having essentially the same biological activity, as a target molecule.

131. A method of stimulating extracellular matrix  
25 synthesis and repair by activation or blockage of an integrin heterodimer comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or of the subunit  $\alpha 10$  thereof, or of a homologue or fragment of said integrin or subunit  $\alpha 10$  having essentially the same biological activity.

132. A method of using DNA or RNA encoding an integrin subunit  $\alpha 10$  or homologues or fragments thereof as  
30 a target molecule.

133. A method according to claim 132, whereby a polynucleotide or oligonucleotide hybridises to the DNA  
35 or RNA encoding an integrin subunit  $\alpha 10$  or homologues or fragments thereof and whereby said polynucleotide or oli-

gonucleotide fails to hybridise to a DNA or RNA encoding  
an integrin subunit  $\alpha 1$ .

134. A method of using a human heterodimer integrin  
comprising a subunit  $\alpha 10$  and a subunit  $\beta$ , or the subunit  
5  $\alpha 10$  thereof, or a homologue or fragment of said integrin  
or subunit having essentially the same biological  
activity, or a DNA or RNA encoding an integrin subunit  
 $\alpha 10$  or homologues or fragments thereof, as a marker or  
target molecule during angiogenesis.

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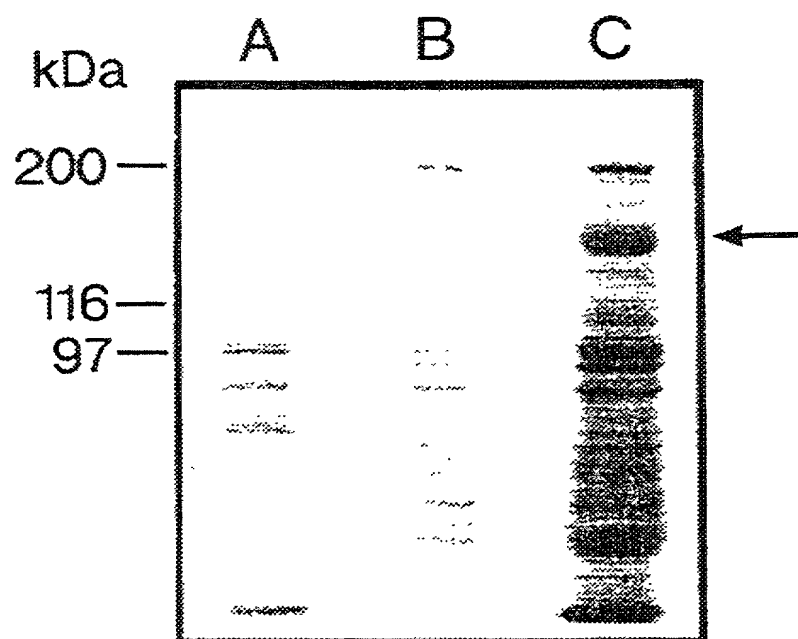


FIGURE 1



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Peptide	Amino acid sequence
1	DNTAQTSA YIQYEPHHSI
2	GPGHWDR
3	AAFDGSGQR
4	FAMGALPD
5	FTASLDEWTTAAR
6	VDASFRPQGXLAP

FIGURE 2

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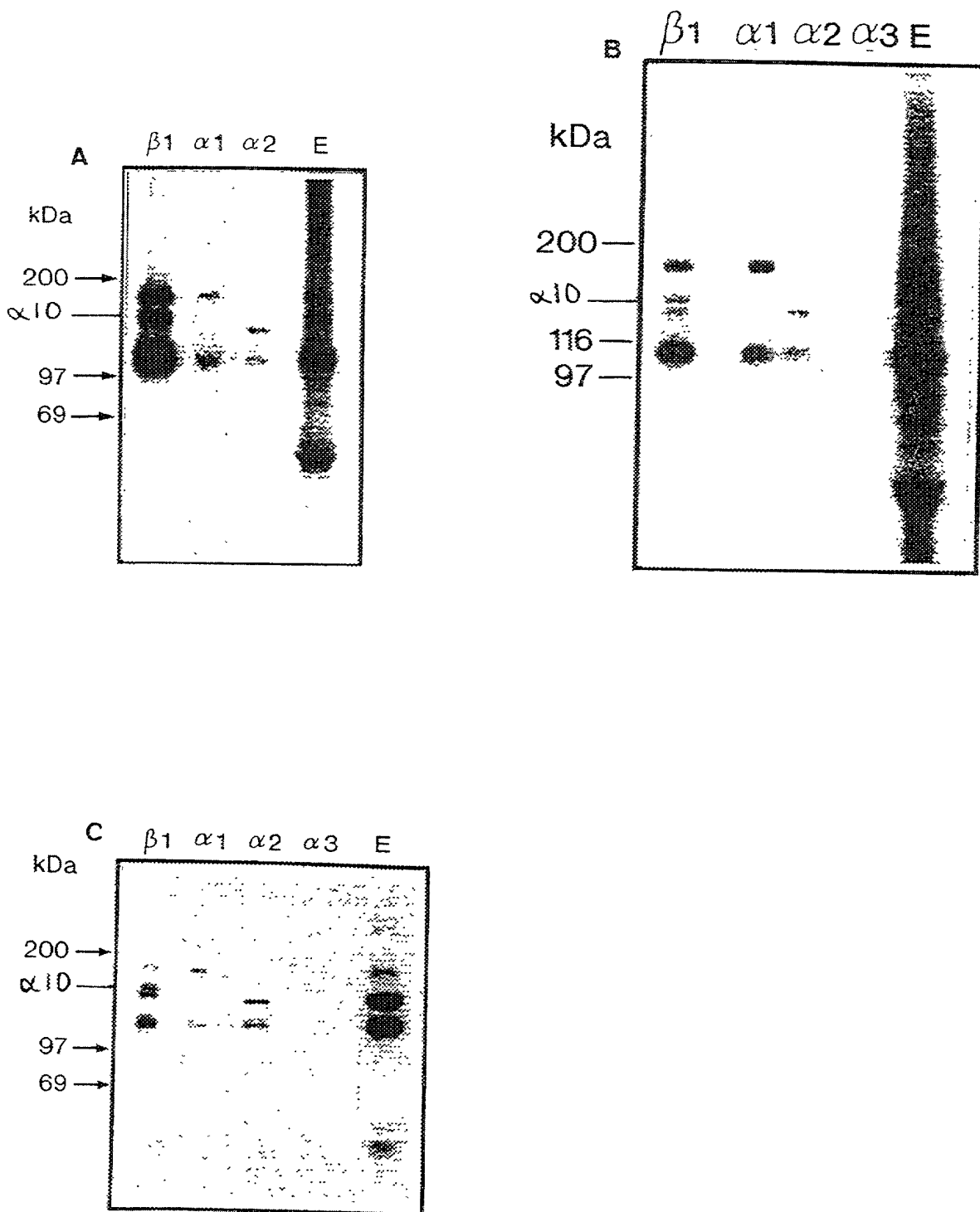


FIGURE 3

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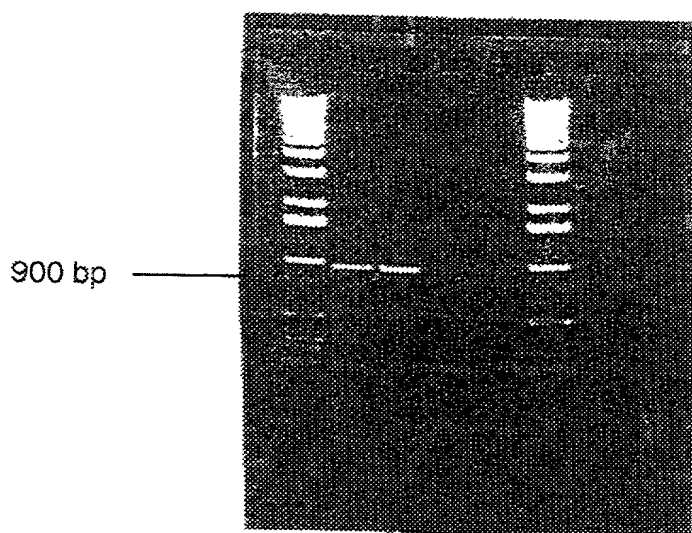


FIGURE 4

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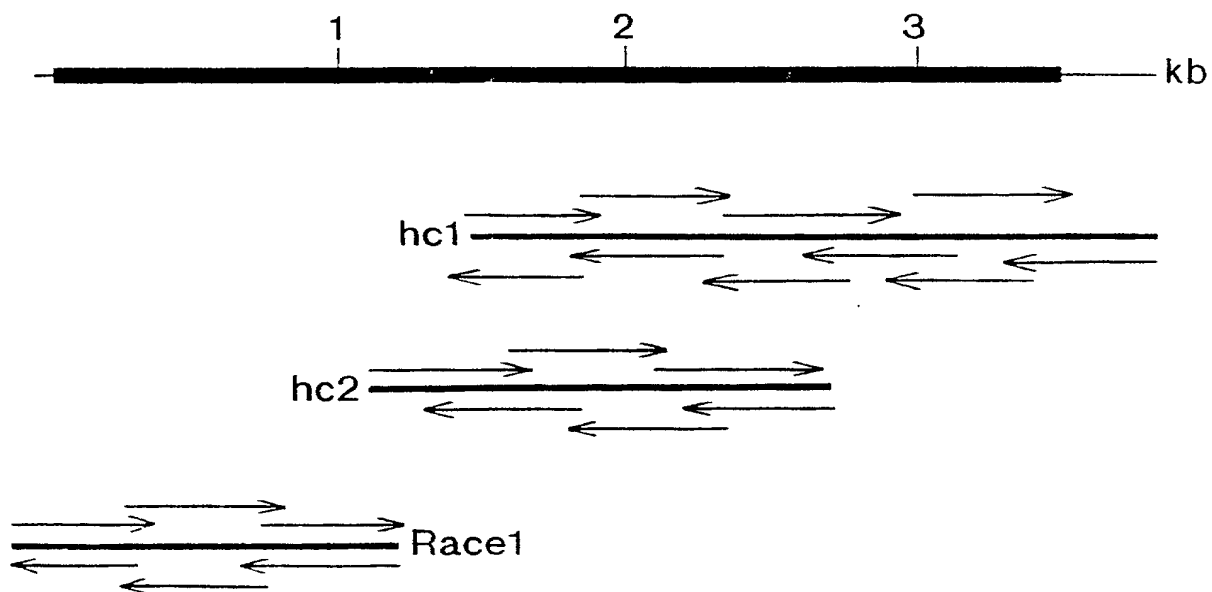


FIGURE 5

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CAGGTGAGAAACGATCAGGATGGAAGTCCCTCTGCTACCTGCTGCTTCTGCCCTGGTGTCTCTGACA	72	CATCTGCCCCAGGATGCTGCTGCTCATGCCATGCCCTCAGCTACTTTGGCCGAATGTGGATGGT	1872
M E L P P V T H L F L P L V P L T	-6	H P A Q R I A A A S M P H A L S Y F G R S V D G	595
GGTCTCTGCTCCCTTTACCTGGATGAACATCACCCAGGCTATCCAGGGCCAGCAGAGCTGAATTT	144	CGCTAGATCTGGATGGATGATCTGGTGGTGGTCTGGGTGCCAGGGGGCAGCCTCTGCTAGC	1944
G L C S P F N L D E H H P R L F P G P P E A E F	19	R L D L D G D D L V D V A V G A Q G A A I L L S	619
GGATACAGTGTCTTACACATGTTGGGGTGGCAGCGATGGATGCTGGTGGGCCCCCTGGGATGGGCT	216	TCGGGCCCCATTTGCTCATCTGACCCCATCTAGGAGTGACCCACAGGCCATCAGTGTGGTTGAGGGGAC	2016
G Y S V L Q H V G G G Q R M H L V C A P W D G P	43	S R P I V H L T P S L E V T P Q A I S V V Q R D	641
TCAGCGACCGAGGGGGAGCTTTATCGCTGCCCTGAGGGGGGCCCAATGCCCTATGCTCAAGGGC	288	TGTAGCGGGGAGGCGAAGAGCTGTCTGACTGAGCCCTTGTCTCAAGTGACTCCGCTACTCT	2088
S G D R R G D V Y R C P V G G A H N A P C A K G	67	C R R R G Q E A V C L T A A L C F Q V T S R T P	667
CACCTTAGGTGACTACCACTGGGAATTCATCTCATCTGCTGTAATATCAGCTGGGATGCTCTGTTA	360	GGTGGTGGATCACCAATCTACATGAGGTTACCCCATCTGATGAATGAGCTGGGGACGCTCA	2106
H L G D Y Q L G N S S H P A V N M H L G M S L L	91	G R M D H Q F Y M R F T A S L D E M T A G A R A	691
GAGACAGATGTCATGGGGATTCATGGCTGTGCCCTCTCTGCTCTGCTGTTGGGAGCTGTGCTTC	432	GCATTTGATGCTCTGGCCAGAGGTTGTCCCTCGGAGGCTCCGGCTCAGTGTGGGAATGTCACTGTGAG	2232
E T D G D G G G P M A C A P L M S R A C G S S V F	115	A F D G S G Q R L S P R R L R L S V G M V T C E	715
AGTTCGGGATATGCTGGCTGTGATGCTTCATTCAGGCTCAGGAGGCTGGGAGCTGCTGCTGCAAGC	504	CAGCTACACTTCCATGCTGGATACATGATTAACCTCGGCGAGTGGCTTCACTGTGACCTTGCCTTC	2304
S S G I C A R V S A S F O P Q G S L A P T A	139	C H F H V L D T S D Y L R P V A L T F A L	719
TGCCACATACATGATGTTGCTTATGCTGCTGAGCTGCTCAACAGCATCTACCCCTGGTCTGAAGTTAG	576	GACAATCTACAAAGCGGGCTGTGCTGAATGAGGGCTACCCACCTCTATACAAAGTGGTCCCTTC	2376
C P T Y H D V V I V L D G S N S I Y P M S E V Q	163	D N T T K P G P V L N E G S P T S I Q K L V P F	763
ACCTTCTAGCAAGCTGGTGAAGAACTGTTATTGACCGAAGACGATACAGTGGGAGTGGATGAT	648	TCAGAGATGTGGCTGACAAATGAATGTGTACAGAGCTGGTGGTTCAGTGATATGAGCATCAGAGC	2448
T F L R R L V G K L F I D P E Q I Q V G L V Q Y	187	S K D C G P D N E C V T D L V L Q V H M D I R G	787
GGGAGAGCCCTGTACATGATGCTGCTCGGAGATTTCCGACGAGGAGGAGTGGTGGAGGAGCAAG	720	TCAGGAGGCGCCCATTTGTGGTTCGAGGTGGCGGGGGAAGTGTGGTATCTACACCTCTGGAGACAGA	2520
G E S P V H E W S L G D E R T K E E V V R A A K	211	S R K A P F V V R G G R K V L V S T T L E W R	811
AACCTGCTGGCGGGAGGAGGAGAGAAACAAAGCTGCCCAAGCAATAGTGGCTGACAGAGAGGTTTC	792	AGGCAAAATGCTTACAAATAGCGCTGAGTATCTCTCTAGAAAGCTCCAGCTGGGCACTCACTCT	2592
M L S R R E G R E T K T A Q A I H V A C T E G F	235	K E N A Y N T S L S I I F S R N L H L A S L T P	835
AGTCAGTCCATGGGGGCGAGCGAGCTGCCAGCTACTGCTGGTGGTGTCTGATGAGGAGTCCATGAT	864	CAGGAGAGAGCCCAATAGGCTGGATGTGGCGCCCTTCTGCTCATGCCCGCTCTGAGTGTGGGGAT	2664
S Q S H G G R P E A A R L L V V V T D G E S H D	259	Q R E S P I K V E C A A P S A H A R L C S V G H	859
GGAGAGGAGCTCTCTGAGCACTAAGGCTCTGAGGCTGGAGCTGACGCTATGGGATTCAGCTCTT	936	CCTGTCTCAGAGCTGGAGCCAGGTGACCTTCTGCTAGAGTTGAGTTAGCTGCTCTCTCTGAGC	2736
G E E L P A A L K A C E A G R V T R Y G I A V L	283	P V F Q T G A K V T F I L E E F E P S C S S L L S	883
GGTCACTACTCTGGCGGCGAGATCCGAGCTCTTCTGAGGAGGATAGCACTATGATGATGCA	1008	CAGGTCTTGGAGCTGACTGCGAGGAGTACAGCTGGAGAGAAATGGCCCTCTCAAGAAACAGCC	2808
G H Y L R R A G C T S S F L R E I R T I A S D	307	O V P G K L T A S S D S L E R N G T L O E M T A	907
GATGAGGATTTCTTCAATGTCACAGATGAGGCTGCTGACTGACATGTCAGTATGAGGATGAG	1080	CAGCTCAGCTACATCCATATGAGCCGCTCTCTGCTAGTGAATGAGCTGAGCTGAGCTGAG	2880
D E R F F F N V T D E A A L T D I V D A L G D R	331	Q T S A Y I Q Y E P H L L P S S E S T L H R Y E	931
ATTTTGGCTTGAAGGCTCCATGCAAGAAACGAAGCTCTTGGCTGGAAATGCTCAGATGCTTTC	1152	GTTCAACCATATGGGAGCTCCAGTGGGTCTGGCCAGAAATCAAAACCATCTCAGGGTTCAGAGCTA	2952
I F G L E G S H A E H E S S E P G L E H S Q I G F	355	V H P Y G T L P V G P G P E F K T T L R V O N L	955
TCCTACTCTGCTTAAAGGATGGATCTTTTGGGATGGGGGCTATGACTGGGAGGCTCTGCTCA	1224	GGTCTATGTGGTGGCTCATCTCTCAGCCCTCTCTCAGCTGTGGCCCATGGGGCAATTAATTC	3024
S T H R L K D G I L F G M V G A Y D M G G S V L	379	G C Y V V S G L I I S A L L P A V A H G G N Y F	979
TGGCTTGAAGGAGCCAGCTCTTCCGCCAGCAATGGCACTGGAGAGGATTCGCCCTGCACTGAG	1296	CTATCAGTCTCAAGTCACTCAATGAAGCTGATAGTGGAGAACTGACTGAACCCGAGCCCA	3096
W L E G G H R L F P P R M A L E D E T F P A L Q	403	L S L S Q V I T N H A S C I V O N L E T P P C G	1003
AACCATGAGCTTACCTGGTACTCTGTTCTTCATGCTTTGGGGTGGAGCCGCTGTTTCTCT	1368	CCTGTGATCCAGAGAGCTTCAACACAAACAGAGTGAATGGAGCAATACTCAATGTGAGTGGTGG	3168
N H A A Y L G Y S V S S M L L R G G R R L F L S	427	P V H P E E L Q H T M R L N G S N T Q C Q V V R	1027
GGGCTCTCGATTAGACATGGAGAAAGTCACTGCCCTTCAAGCTTGAAGAGATGGGCTGTGAGGTT	1440	TGCCACTTGGGAGCTGGCAAGGGGAGTGGTCTGTGTGAGCTATTGAGGCTGGTTCACAAATGATTT	3240
G A P R F A H R G X V I A Y Q L K K D G A V R V	451	C H L G Q L A K G T E V S V G L L R L V H N E F	1051
GCCAGAGCTCTCAGGGGAGCAGATGCTTATCTTTGGAGTGGCTGCTGCCATGATGATGATGAG	1512	TTCCGAGAGCCAAAGTTCAAGTCCCTGAGCGGTGGTCAACCTTTGAGCTGGAGACGAGAGGCGATGTC	3312
A Q S L Q G E Q I G S Y F G S E L C P L D T D R	475	F R R A K P K S L T V V S T F E L G T E G S V	1075
GATGGAGCACTGATGCTTACTTGTGGCTGCCCATGCTGCTGGAGCCGCCAGACAGGAAACAGGCT	1584	CTACAGTGAAGTGGCTCCCGTTGGAGTGGAGGCTCTTGGAGTGGTTGAGAGCCGCGCTATCTCTATC	3384
D G T T D V L L V A A P H F L G P Q N K E T G R	499	L Q L T E A S R W S E S L L E V V Q T R P I L I	1099
GTTTATGTATGTCAGGAGGAGCTCTGCTGAGCTTCAAGGAGCACTTCAAGGAGGAGGAGGAGGAG	1656	TCCCTGTGGATCTCATAGGAGTGTCTGGAGGAGTGTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG	3456
V Y V Y L V G Q Q S L L T L O G T L O P E P P Q	523	S L M I L I G S V L G G L L L L A L L V F C L W	1123
GATGCTGGTGTGCTTGGCATGGAGCTCTCTGATCTGAGCAAGATGGTTTGTGATGTGGCTGTG	1728	AAGCTGGCTCTTTGCCCATAGAAATCCCTGAGGAGAGAAAGAGAGAGAGAGAGAGAGAGAGAGAG	3528
D A R F G F A M G A L P D L N O D G F A D V A V	547	K L G F P A H K K I P E E K R E E K L E O	1145
GGGCGCTCTGGAGATGGCCAGGAGGAGCTTACCTGTACATGAGCCAGAGTGGAGTGGAGGCGC	1800	TAGAAATAGGCTTAGAAATGCTCCCTGGAGCTTCTTCAAGAGCTTGCATAAAGCAGAGGTTGGG	3600
G A P L E D G H Q G A L Y L Y H G T O S G V R P	571	GCTCAGATGGAGAGAGGCGCTCTGGATATCTCCAGAGCCAGAGGCTGACTTGACTTTTGGTCT	3672
		AGGATGCTCTGCTGAGAGTGGGCTTACTCTCAGAGAGAGAGAGTGGACCAAACTAGCCTGCTCC	3744
		CAGCTCTGCTTCCCTCTCTGATGCTGCTGCTCATAGCCAGAGCTGGGCTTTTGTGGGCTCTTT	3816
		TATCCCCAGATCAATATTTTGTGCTAGGAAAAAAGAGGCGCCGGAATTCATATCAAGCT	3888

FIGURE 6

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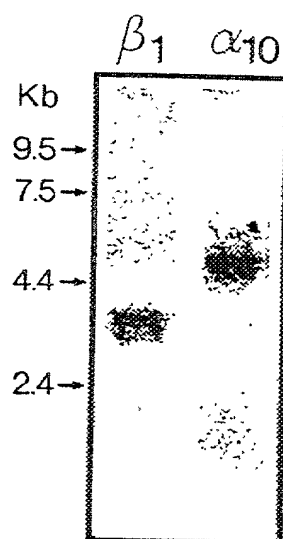
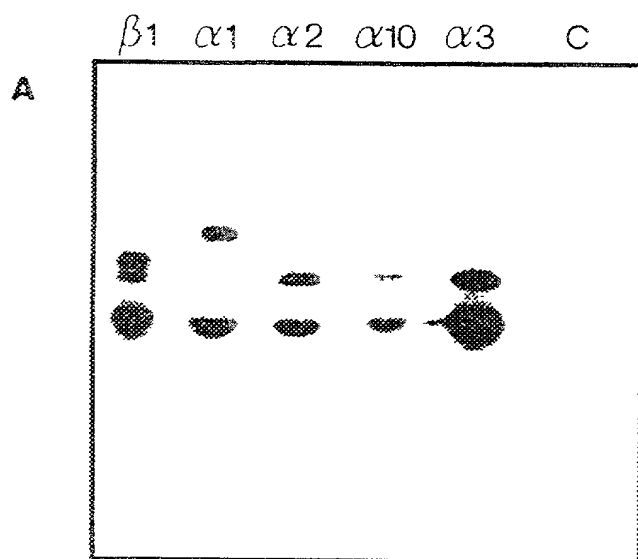


FIGURE 7

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**B** IP:  $\alpha 10$   $\beta 1$   
 Blot:  $\beta 1$   $\beta 1$

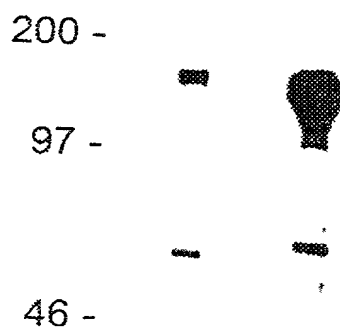


FIGURE 8

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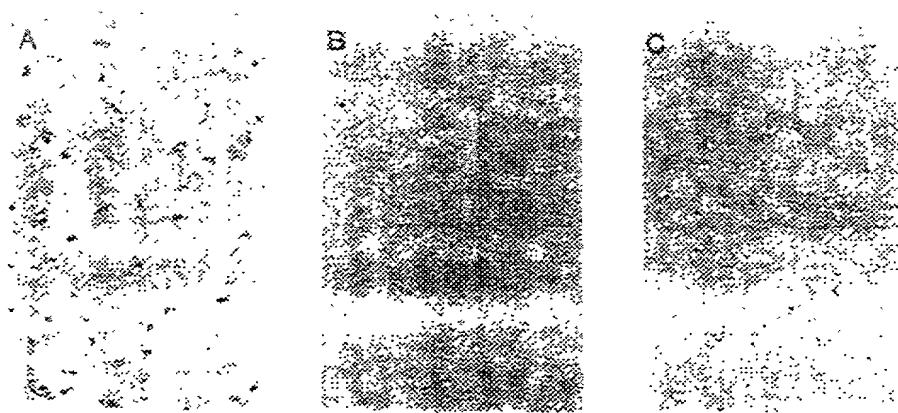


FIGURE 9



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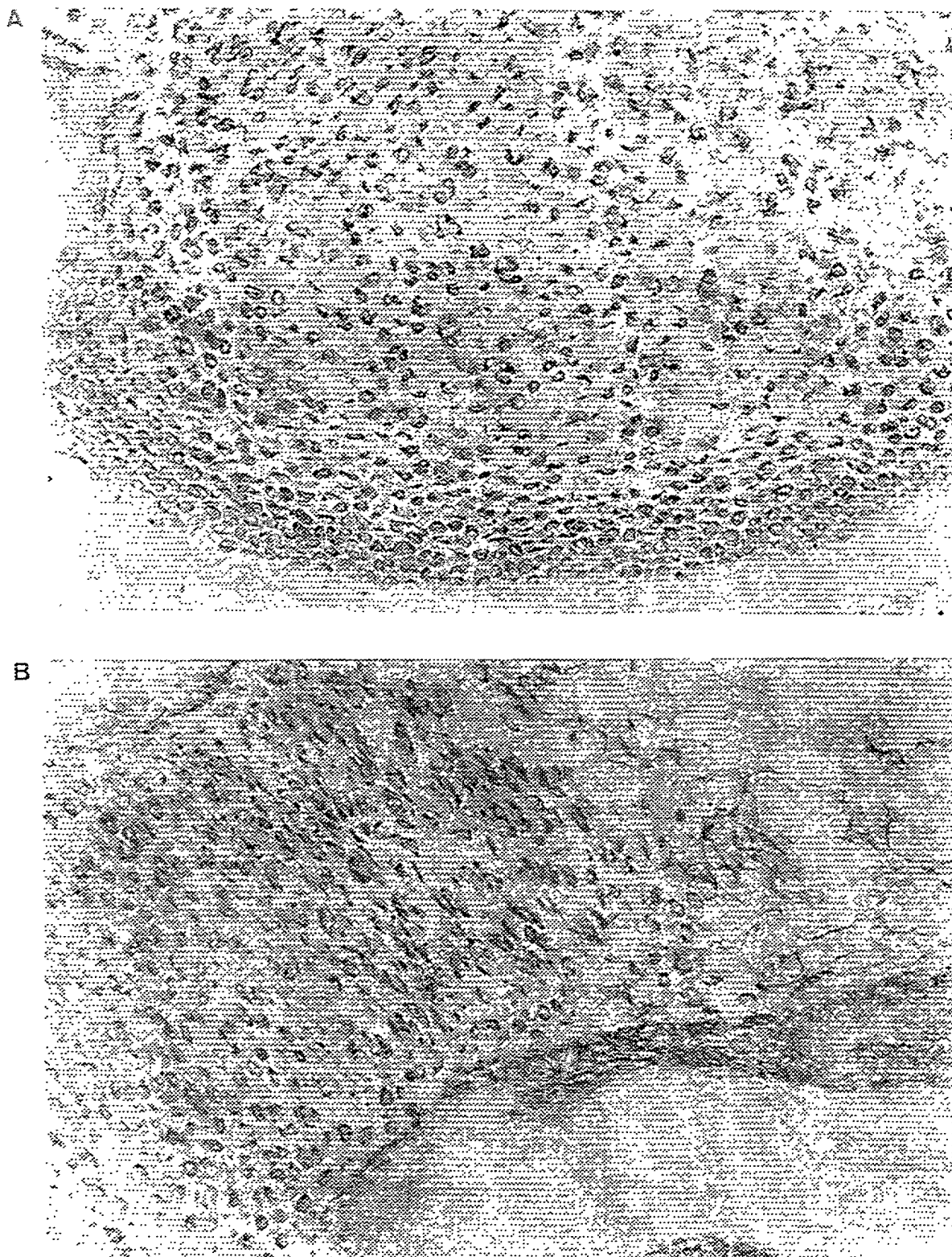
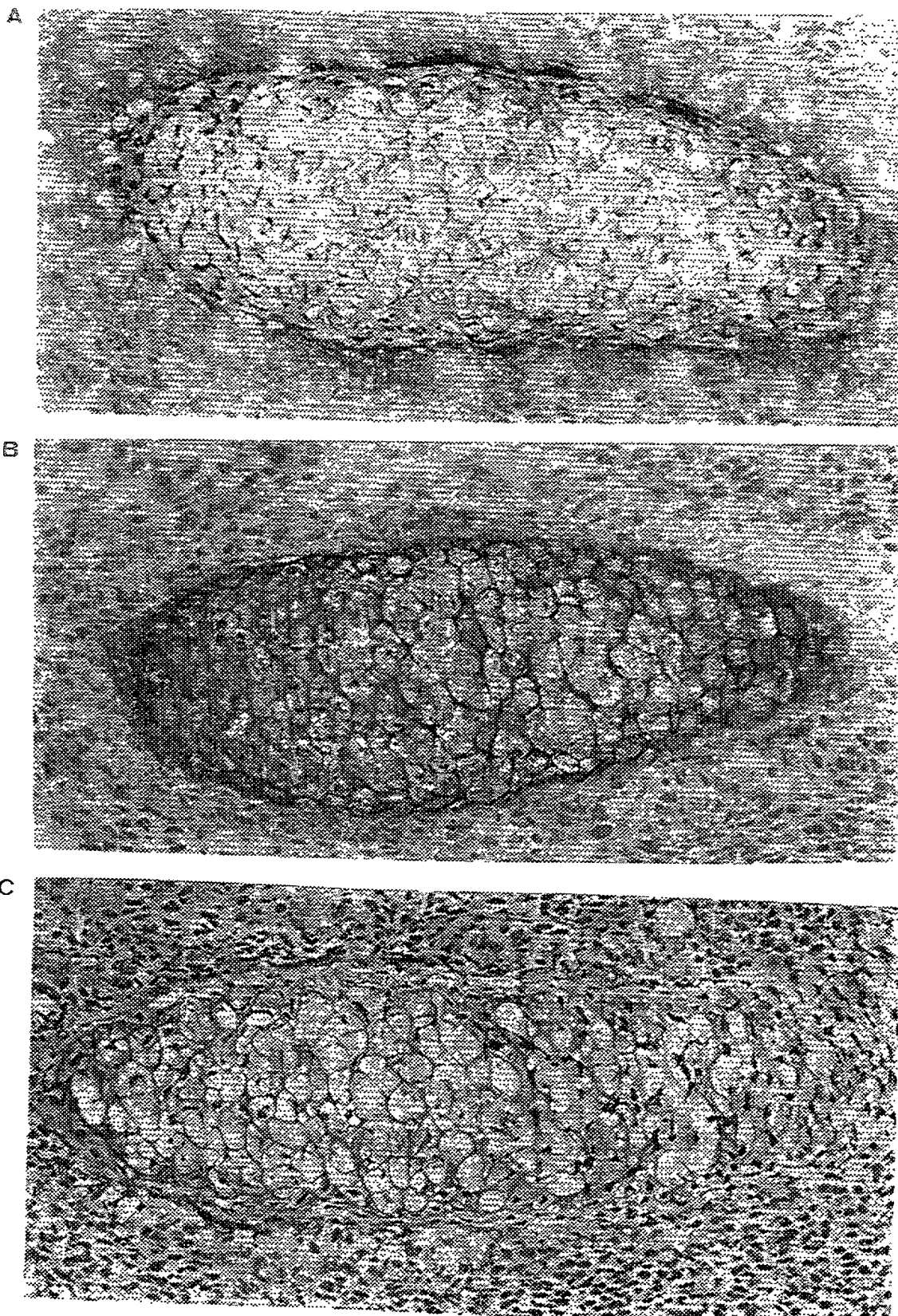


FIGURE 10

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**Human RNA Master blot**

Tissue	$\alpha 10$ expression	Tissue	$\alpha 10$ expression
Aorta	++++	Thyroid gland	-
Trachea	+	Salivary gland	-
Lung	++	Spleen	-
Fetal lung	++	Fetal spleen	-
Kidney	++	Thymus	-
Fetal kidney	(+)	Fetal thymus	-
Heart	(+)	Peripheral leucocyte	-
Fetal heart	++	Lymph node	-
Spinal cord	++	Appendix	-
Mammary gland	(+)	Placenta	-
Bone marrow	(+)	Whole brain	-
Small intestine	(+)	Fetal brain	-
Skeletal muscle	-	Amygdala	-
Liver	-	Caudate nucleus	-
Fetal liver	-	Cerebellum	-
Colon	-	Cerebral cortex	-
Bladder	-	Frontal lobe	-
Uterus	-	Hippocampus	-
Prostate	-	Medulla oblongata	-
Stomach	-	Occipital lobe	-
Testis	-	Putamen	-
Ovary	-	Substantia nigra	-
Pancreas	-	Temporal lobe	-
Pituitary gland	-	Thalamus	-
Adrenal gland	-	Subthalamic nucleus	-

FIGURE 12

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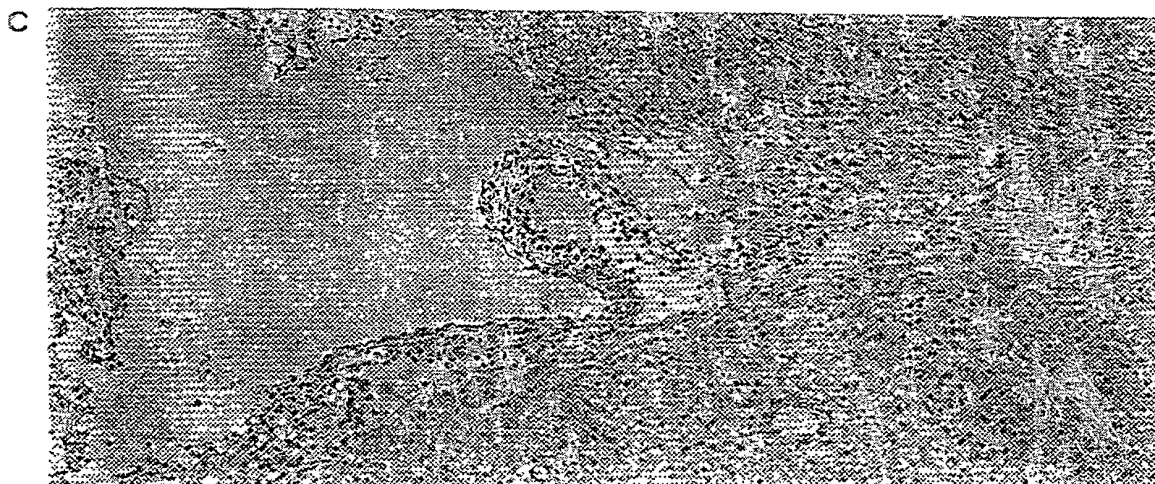
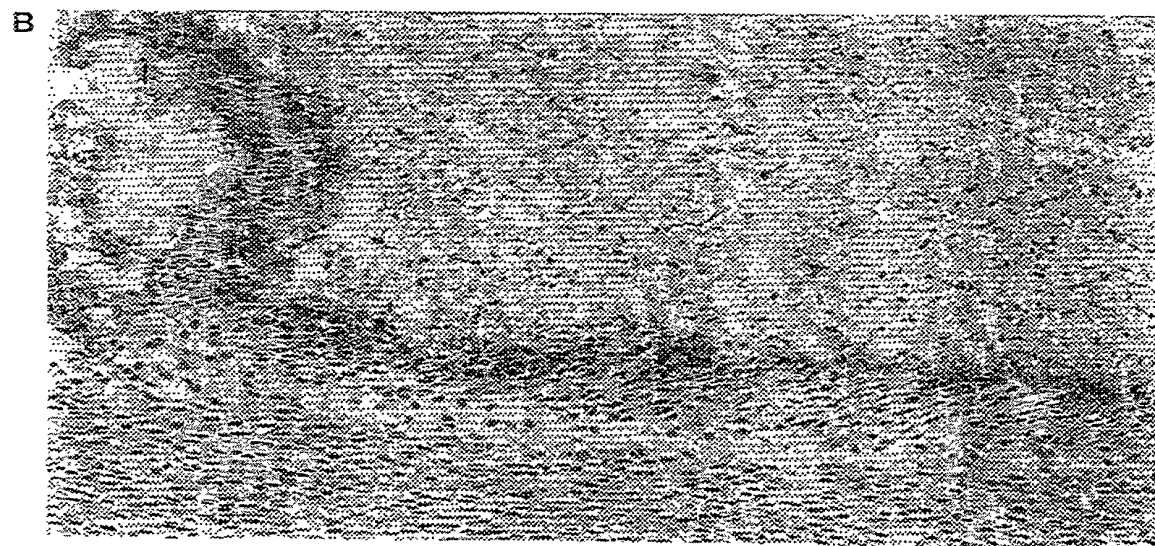
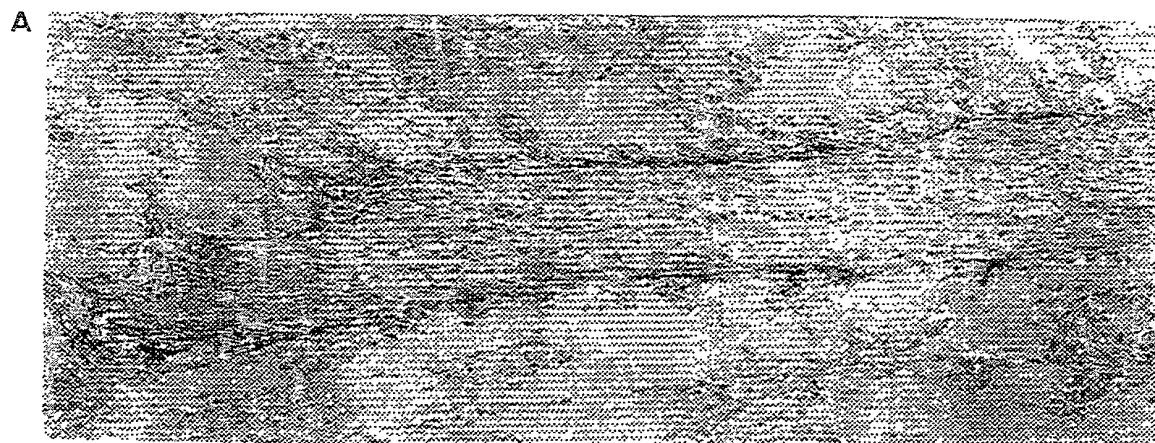


FIGURE 13

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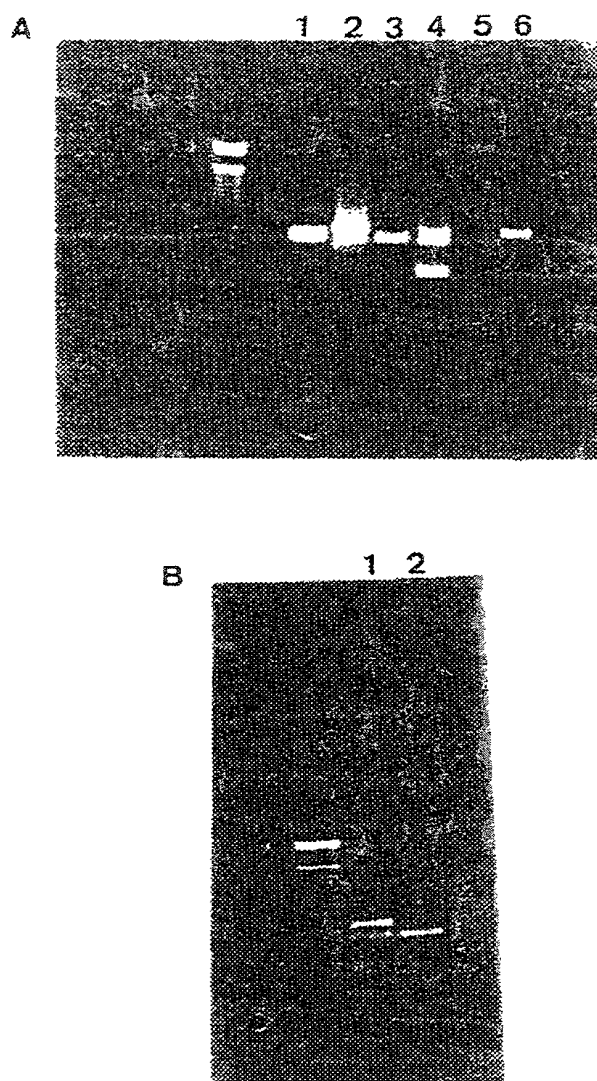


FIGURE 14



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TGNTHMMKCMCAGAKMGWSAKGNCCGAKGGTKGKGVAAVGTGACARAGCTNGMNAARANGAAGTATGACCCWGTGGGC 80  
 ? ? ? ? R ? ? ? P ? V ? ? ? D ? A ? ? K ? ? K Y D ? W A  
 V ? ? ? ? H ? ? ? ? R ? ? ? ? V T ? L ? K ? ? S M T ? G  
 ? ? ? ? T ? ? ? ? ? G ? ? ? ? . Q S ? ? K ? ? E V . P V G  
 CRAGATAGMKAMDAAGCNGMSAGKTRAMGGACGATGGNCCMGCCAAVCGABWGGNAHTBCGGCNWDCARNGTCCAAATK 160  
 ? I ? ? K ? ? ? ? G R W ? ? Q ? ? G ? ? G ? ? Q ? P N  
 P R . ? ? ? S ? ? ? ? D D G P A ? R ? ? ? ? A ? ? V Q ?  
 ? D ? ? ? A ? ? ? . R T M ? ? P ? ? ? ? ? R ? ? ? S K ?  
 SANKTSCAGGAACCMACGGAMTGGCTCGCARCCCDTAGGGATCAGGKACGATGRCTSCCCGRNSKACTCSGNKTGATWA 240  
 ? ? ? R N ? R ? G S Q P ? G I R ? D ? S P ? ? S ? . ?  
 ? ? ? G T ? ? G ? A R ? ? P . G S G T M ? ? R ? ? T ? ? D ?  
 ? S Q E P T ? W L A ? ? R D Q ? R ? L ? ? I  
 ATCGMNGWTMGGMAGGCGGCGGGAATTRWAAAGTANTGGTNGAMAKATGNGVHGGAWATGATRRGTMGACTVTMVGGVAK 320  
 I ? ? R ? A ? E L ? S ? G R ? M ? R ? . ? V D ? ? G ?  
 S ? ? V G R R R R N ? K V ? V ? ? ? ? G ? D ? ? T ? ?  
 N R ? ? ? G G G I ? K ? W ? ? ? ? ? ? M ? ? R L ? R ?  
 VTAKSGGTACAGGCGAAKACARGRAKGTGTCTGAGGAADTCAGNAGGACAAMMTTGGCGAAGTCMGGACTTAGKATRGAT 400  
 ? ? Y R R ? Q ? ? V . G ? Q ? D ? ? A E V R T . ? ?  
 ? ? G T ? ? G ? V S E E ? ? R T ? L P K S G L ? ? D  
 ? ? V Q A ? T ? ? C L R ? S ? G Q ? C R S ? D L ? ? I  
 ACGAANCCTRGATCTTAMADGGGGGNKAGCGAGTGCSTAAACGVARATRGNSWGTCTACTTMAACNCCAAGNGDGGACA 480  
 Y E ? ? I L ? G G ? R V ? K R ? ? ? ? L L ? ? Q ? ? T  
 T ? ? ? S ? ? G ? S E C ? N ? ? ? ? V Y ? N ? K ? G H  
 R ? ? D L ? ? G ? A S A . T ? ? G ? S T ? T P ? ? D  
 TTTACTAGASGAGGAGAGTAGCCAGATCACDTGAGATGATCTAAKGTGGGGTCCCGTTGCCAGTATATGAGAGGACTGGT 560  
 F T R ? G E . P D H ? R . S ? V G S R C Q Y M R G L V  
 L L ? E E S S Q I T . D D L ? W G P V A S I . E D W  
 I Y . ? R R V A R S ? E M I . ? G V P L P V Y E R T G  
 TCGGCAGACATWGATGCTCTTTGCTGACTCACATATTGTTGCCVTGAGKATGATCAGATACGATCTGTGTGCTCCCTCATCA 640  
 R Q T ? M L F A D S H I V A ? ? M I R Y D L ? S L I  
 F G R H ? C S L L T H I L L P . ? ? S D T I ? C P S S  
 S A D I D A L C . L T Y C C ? E ? D Q I R S ? V P H H  
 TGAATSTGRGCCGTGATGCTAATGAGATTGCGCTATGATGGAACAAGAGACTTMTGCTACAGCAGGCGAATGAAGGTTTC 720  
 M N ? ? R D A N E I R L . W N K R L ? L Q O A N E G F  
 ? ? ? A V M L M R F A Y D G T R D ? C Y S R R M K V S  
 ? ? ? P . C . . D S P M M E Q E T ? A T A G E . R F  
 TAGAGTAGGAGTCTCAGGAGGAGAGAACTGTGGACCTGGAGGACCAGGGACTCCAGGAGGAAGTWGCCAACACTGGCTT 800  
 . S R S L R R R E T V D L E D Q G L Q E E V A T T G L  
 R V G V S G G E K L W T R D S R R K ? P Q L A  
 L E . E S Q E E R N C G P G G P G T P G G S ? H N W L  
 GMAGTTTCCGGCTCCGATCCTGATACWGGCTCGTCCTTVGAGTTATCCCTCTCTTGCTGGATGGCTCAGAAATGCCTGG 880  
 ? F R L R S . Y ? L V L ? V I P L S C W M A Q K C L  
 ? S F S S D P D T G S S ? E L S P S L A G W L R N A W  
 ? V S G A I L I ? A R P ? S Y P P L L L D G S E M P G  
 ACCTTTTCATCCCCACTGGACAACTAGGCGTCTGGCGTTGTGGCCCTGGGATTGTGGGGCTGTGTGCCCTCATATCCTC 960  
 D L F I P T G Q T R R L A L W P W D C G A V W P H I L  
 T F S S P L D K L G V W R C G P G I V G L C G L I S S  
 P F H P H W T N . A S G V V A L G L W G C V A S Y P  
 CATTCTGTCTATTCTCACCCTAATCTGTCCCTGGNTACGACTCAAGCCCYGACTGACAHTGTGGTACAAGATAAGGAGGG 1040  
 H S V Y S H P N L S L ? T T Q A ? T D ? V V Q D K E G  
 I L S I L T L I C P W ? R L K P ? L T ? W Y K I R G  
 P F C L F S P . S V P G Y D S S P D . ? C G T R . R G  
 AGCCCCAGGTGGGTGAGATGGAAGCTGAGATGGTNCACCTGTGTGCCACCTCATTGTAATCAACTNCCTTGACTGAAGTT 1120  
 A Q V G E M E A E M V H C V P T S L . F N ? L D . S  
 E P R W V R W K L R W ? T V C ? P H C N S T ? L T E V  
 S P G G . D G S . D G ? L C A ? L I V I Q L P . L K L  
 AAAATCCAGATCCYTAGGSATGAGGGGAAGAACCTGCCAAGACGGGTGAGGAAGGCAGTGCTAAGGGAAGGCTCCTGCA 1200  
 . N P D P . G . G E E P A K D G S G R Q C . G K A P A  
 K I Q I ? R D E G K N L P E T G Q E G S A K G R L L Q  
 K S R S L G M R G R T C R Q R V R K A V L R E G S C  
 GGCCTCTGCACTGGACTTCATTGAGTCCCATGCAATCTCATAGCTCTTCCCYTATCTCTGTCTTGAGTCTAG 1280  
 G L C S W T S F S P I A R I S . L F P L S L C L E S S  
 A S A V G L H S V P L P E S H S S S ? Y L S V L S L  
 R P L Q L D F I Q S H C Q N L I A L P ? I S L S . V .  
 TTAAGAATTTGTTACCGGAGACAGAATTCTCTTTCTAGCCTCCTGGCCAGATATTTAAAAGGAGGGGGTGGGTTACTT 1360  
 . E F V T G D R I L F L S L L A R Y L K G G G W V T  
 V K N L L P E T E F S F L A S W P D I . K E G G G L L  
 L R I C Y R R Q N S L S . P P G Q I F K R R G V G Y F

FIGURE 15a

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FIGURE 15b

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CAGGTGAGGGGAAGCAAACCTTGGTTTCTGCTGGGAATGGAAGTTATGTGGATTGTTTATAATTGGGACCATTATGGCTAAA 2800  
 R . G K Q T W F L L G M E V M W I V Y N W D H Y G .  
 T G E G S K L G F C W E W K L C G L F I I G T I M A K  
 Q V R E A N L V S A G N G S Y V D C L . L G P L W L K  
 ATCTYGGCGGGCTCAGGTGGGAGGTTAATACCGATGCTATATTTCTGTGTGCACTCATGTTCTTAGACACCCAAATGG 2880  
 N L A G A Q V G G . Y R C Y I S C V H S C S . T P K W  
 I ? R A L R S E V N T D A I F P V C T H V L R H P N G  
 S ? G R S G R R L I P M L Y F L C A L M F L D T Q M  
 CAGTGGCCAAAACCTTCTCTGGCTTGTACCTCATTATCTAAACCTTTGTACCTAATTATCTAAAACCTTGGTCTTAAACT 2960  
 Q W P K L P L A C T S L S K P L Y L I I . N L G P K L  
 S G Q N F L W L V P H Y L N L C T . L S K T L V L N  
 A V A K T S S G L Y L I I . T F V P N Y L V P W S . T  
 CCACAGACATGAGGGCAGAAAAGAGACGTGTCTCTCATCTTCCATTCCGTTACAGTATCCTACCTTCCCTGCTTCT 3040  
 H R H E G T E K R R V S H L P F G Y T D S Y L P C F  
 S T D M R A Q K R D V S L I F H S V T L I P T F P A S  
 F Q T . G H R K E T C L S S S I R L H . F L P S L L L  
 CCCTGCTATTGGTGCTCCTTGGTGCTGAGGCATAATTGCCTTACTATGTGGTCAGAACTCTGGGTTCCGCTAACGACCG 3120  
 S L P L V L L G A . G I I A L L C G Q N S G F A . R P  
 P C H W C S L V P E A . L V Y Y V R T L G S P N D R  
 P A I G A P W C L R H N C L T M W S E L W V R L T T  
 AGCTACAGTTTCTGGTCTCAGCCCTGCCAATTTCTGGATTAAAAAAGGCTCAGATATAAAATACCTTTTCTGA 3200  
 S Y S F W S H S P A N F L D . K K K A H I . N T F S E  
 A T V S G L I A L P I S W I K K K R L T Y K I P F L  
 E L Q F L V S . P C Q F P G L K K V G S H I K Y L F .  
 AAATGAGCACAGTGTGAGTTGAAGTTAGATTTTGGGGGATGGAGGTTGCTTGGATGCAAAGAGCAAGACAGTAGAGAAG 3280  
 N E H S V S . S . I L G D G G L L G C K E Q D S R E  
 K M S T V . V E V R F W G M E G C L D A K S K T V E K  
 K . A Q C E L K L R D F G G W R V A W M Q R A R Q . R R  
 AGAATCATGGGAGGGATAAGAGGCTGGAATTTTCCCTGCTAGTGCCCTATAATCTTTCTTCTTAAATAACAGCTCTG 3360  
 E N H G R D K R L E F F P A S A L . S L F P K I T A L  
 R I M G G I R G W N F S L L V P Y N L C F L K . Q L  
 E S W E G . E A G I F P C . C P I I F V S . N N S S  
 ATTTTATGGGAATTGGGGTCAAGAGAAAGGAATCAGTAGGCACAGATGGGACCCCAAGCGTGGACTAAAGTTTGAGSAAA 3440  
 I L W E L G S G E P N Q . A Q M G P Q A W T K V . G N  
 F Y G N W G Q E K G I S R H R W D P K R G L K F E E  
 D F M G I G V R R K E S V G T D G T P S V D . S L R K  
 CTATGGGAGTAGGCAAGGGGTGTTTGTAAAGTGGATGAGATGAGGAGATTGTGGTGGGGGGAGTCTTGGGGGTGATAGG 3520  
 Y G S R Q G V F R W M R . G D C G G G E S W G .  
 T M G V G K G C L . G G . D E I V V G G S L G G D R  
 L W E . A R G V C K V D E M R R L W W G G V L G V I G  
 ACCCTTAACAGGGATAGATGGCAAACCTGTGTGTGGGCAGGCGGGTGGTCCACCCACTTAATTAGCGTTGAGGTTGGCAG 3600  
 D P . Q G . M A N C V W A G R W F H P L N . R . G W Q  
 T L N R D R W Q T V C G Q A G G S T H L I S V E V G R  
 P L N T G I D G K L C V G R P V V P P T . L A L R L A  
 GGCTGGAAGGAGCCAGCACTCTCAACCTTGGAGAAAGTGAAGTGTGACAAGAAGAAACAGAAAGAGGAGACACCGGGGC 3680  
 G W K E P A L S T L E K V Q V . Q E E T E R G D T R A  
 A G R S Q H S Q P W R K C K C D K K K Q K E E T P G  
 G L E G A S T L N L G E S A S V T R R N R K R R H P G  
 AGGGAGCTCCTTGCCATCGTTTCTTCCCATGGCCCTGGCTTGGGAAGAATTAGGAAAGGGTGGTGACTCTGCATCCTCA 3760  
 G S S L P S F L P M A L A L G R I R K G W . L C I L  
 Q G A P C H R F F P W P W L W E E L G K G G D S A S S  
 R E L L A I V S S H G P G F G K N . E R V V T L H P Q  
 GAAAAGCCCTCTCTCCCTCTTTGGACTCTCGAGGCTTAGAGAGGAGAAATGTGTAGGAGGAATGATGTGGAAGAGTAAC 3840  
 R K A L S P S L D S R G L E R R M C R R N D V E R V T  
 E K P S P L S L W T L E A . R G E C V G G M M W K E . L  
 K S P L S L F G L S R L R E E N V . E E . C G K S N  
 TGACCTATCCAGATGTGTCTGTGAATTCAGGAATGAGAATGGAATACAGCTGTGCTTCAAGATGGCCGAGGGC 3920  
 . P I Q M C L . M R F Q E . E W K Y S C A S A W P R A  
 D L S R C V C E . D F R N E N G N T A V L Q H G R G  
 L T Y P D V S V N E I S G M R M E I Q L C F S M A E G  
 CTTAGGATCCCTCACCCCCACCCACAGGAAGAGAAATCATCCAATCATCCACCTGGGCTTCTGAGGACATGACATTGAC 4000  
 L G S L T P T P O E E N H P I I P F G V L R T . H .  
 P . D P S P P P H R K R I I Q S S H L G F . G H D I D  
 L R I P H P H P T G R E S S N H P T W G S E D M T L T  
 ACAGAGCAGGAGAGCTGAGATAGAAACACTCCCTCTGTCTTGTCTCCCACTAAGCCTCACCAGTCCTTCATTAACGTAT 4080  
 H R A G E L R . K H S L L S C L P L S L T S P S L T D  
 T E Q E S . D R N T P S C L V S H . A S P V L H . L I  
 Q S R R A E I E T L P P V L S P T K P H Q S F I N .

FIGURE 15c



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TGGTGGATGCTAATTATGATCCTCACCCCTCAGGTCTCTGCTCCCCCTTTAATCTGGATGAACACCCACGACTCTTC 4160  
W W M L I M I L T P Q V S A P P L I W M N T T H D S S  
G G C . L . S S P L R S L L P L . S G . T P P T T L F  
L V D A N Y D P H P S G L C S P F N L D E H H P R L F  
ACAGGGCCACCCAGAGGCCGAATTTGGATACAGTGTCTTACAGCATGTTGGGGGTGGACAGCGATGGTGAGAGGGGAAAACA 4240  
Q G H Q R P N L D T V S Y S M L G V D S D G E R E N  
H R A T R G R I W I Q C L T A C W G W T A M V R G K T  
T G F P E A E F G Y S V L Q H V G G G Q R W . E G K Q  
GAGGACCGTGGGACTATGCACTCACTGATAAAGGGGAGGACCGGTCCAAGCTGGCCTTTGAAAGTGCTGGGGC 4320  
R G P W D R D Y A L T D K G E D R S K L A F E S A W G  
E D R I G T M H S L I K G R T G P S W P L K V P G A  
P T V G S G L C T H . . R G G F V Q A G L . K C L G  
TCCATGACGTCTCATGCACTCTCCCTCTCACTATACTAAGGACCATGCTCACCGGATCTTTATATCCATATTCTCCTTCC 4400  
S M T S H A L S L S L Y . G P C S P D L Y I H I L L P  
P . R L M H S P S H Y I T R D H A H R I F I S I H I L L P  
L H D V S C T L P L T I L T R T M L T G S L Y P Y S P S  
AGGATGCTGGTGGGTCGCCCTGGGATGGGCCATCAGGTGACCGGAGGGGATGTTTATCGTTGCTCTATAGGGGGATT 4480  
G C W W V P P G M G H Q V T G E G M F I V A L . G D  
Q D A G G C P L G W A I R . P E R G C L S L L Y R G I  
R M L V C A P W D G F S G D R R G D V Y R C S I G G F  
CCACAGTGCTCCATGTACCAAAGGCCACCTGGGTAAGAAGAAGCCTGACCTTTCCCCTGCTAATTCCTGATGTTGACATC 4560  
S T V L H V P K A T W V R R S L T F P P L L I P D V D I  
P Q C S M Y Q R P P G . E E A . P F P C . F L M L T S  
H S A P C T K G H L G K K K P D L S P A N S . C . H  
TAGTAAGTCTGACCCCTTGGACCTTGTCTTCAATGACCTGAAGAAAGCGAAGTATGACCCCATGACTTCATTCT 4640  
. . L . P L G P C L Q . P . T K E A E L . P H D F I L  
S N S D P L D L V F N D P E L K K P N Y D P M T S F  
L V T L T P W T L S S M T L N . R S R T M T P . L H S  
CTTCTACCCCTTCTCCAACCGGTGACTATCAACTTGGAAATTCCTCTCAGCCTGCTGTGAATATGCACCTAGGGATGTC 4720  
F Y P S S N Q V T I N L E I P L S L L . I C T . G C  
S S T L P P T R . L S T W K F L S A C C E Y A P R D V  
L L F F L Q F G D Y Q L G N S S Q P A V N M H L G M S  
TCTACTAGAGACAGATGCTGATGGGGATTGATGGTGAGCTGAAAGAAGGGCCTCAGAAGGTTACAGCAGGGAAGAGAG 4800  
L Y . R Q M L M G D S W . A E R R A S E G S Q Q G R E  
S T R D R C . W G I H G E L K E G P Q K V H S R E E S  
L L E T D A D G G F M V S . K K E G L R R F T A G K R  
CATTATGGTATCTGGGCGAGTGGCTTGGCCCTTTCATCCAGTGTCTGGAGGCAGAGTCAGGCCTGATCTACAGAGT 4880  
H Y G I W A V V A W A F H P S V L E A E S G L I Y R V  
I M V S G Q W W L G P F I P V F W R Q S Q A . S T E  
A L W Y L G S G G L G L S S Q C S G G R V R P D L Q S  
GAGCTCCAGGACAGCCAAAGGCTATGCAGAGAAACCTGTTTTGAAAAACCCAAAACCAAACAAACAACAAAC 4960  
S S R T A K A M O R N P V L K N P K P K L T K Q Q Q  
A P G Q P R L C R E T L F . K T O N Q N . P N N Q N  
E L Q D S Q G Y A E K P C F E K P K T K T N Q T T T T  
AGAAAAAGCACCGTGGTAAGGGAATTAGTCTGTATAGAAGAGACAAGGAATTCAAAACCTAGAGAGCAAGGCAGGGTT 5040  
Q K K H R G K G N . S V . K R Q G I Q N P R E Q G R V  
R K S T V V R E I S L Y R R D K E F K T L E S K A G F  
E K A P W . G K L V C I E E T R N S K F . R A R Q G  
CCCCATGGAGTGGTCTCCATCTCTCTTTAACTAGGTGTGTGTTCCGAGAGGCCCTCTCAAGCCTGGGGATAACTATTTC 5120  
P H G V V S I S L L T R C V F R E A L S S L G I T I S  
P M E W S F S L F . L G V C S E R P S Q A W G . L F  
S P W S G L H L S F N . V C V P R G P L K P G D N Y F  
TCCTATCCACCCAGGCCTGTGCCCTCTTGGTCTCGTGCTGCGGAGCTCTGTCTTCAGTTCTGGAATATGTGCCCGT 5200  
P I H P G L C F S L V S C L R Q L C L Q F W N M C P  
L L S T Q A C A P L W S R A C G S S V F S S G I C A P  
S Y P P R P V P L F G L V P A A A L S S V L E Y V P V  
GTGGATGCTTCATTCCGGCCCCAGGGAAGCCTGGCACCCACCGCCCAACGTGAGCCAGTGGGAAGGCCCTGGAAGCTCAG 5280  
C G C F I P A P G K P G T H R P T . A S G R A L E A Q  
V D A S F R P Q G S L A P T A Q R E P V E G P W K L S  
W M L H S G P R E A W H P P P N V S Q W K G P G S S  
TTCCAGATAGGGATGCTGGGTGGGAAAAACTAGGACAAAGACTTGGTGGAGGCTGTCATGGCTATCCTCATCATTCCC 5360  
F P D R D A G W E K L G Q R L G G G S A W L S S S F P  
S Q I G M L G K N . D K D L V E G L H G Y P H S  
V F R . G C W V G K T R T K T W W R V C M A I L I I P  
AAGTGTGCTTGCAAGAGGCTCCTGTTTGCTAACTGATTAGAATTCAGACTCCTTAGGAGAGCCTCAAGACACCAGGAT 5440  
S V L A E E A P V C . L I R I Q T P . E S L K T P G  
Q V C L Q K R L L F A N . L E F R L L R R A S R H Q D  
K C A C R R G S C L L T D . N S D S L G E P Q D T R I

FIGURE 15d

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CTGGTTTACCAACTTAAAAACAAAACAAAACAGCATATCCTGTGCACAGCCTATCCCTCATCCATCAGGTGCTCCTCCAT 5520  
 S G F T N L K T K Q N S I S C A Q P I F H P S R V L H  
 L V L P T . K Q N K T A Y P V H S L S L I H H V S S I  
 W F Y Q L K N K T K Q H I L C T A Y P S S I T C P F

ATCTTATTTTGTGGGTCTTATAGATGCCAAGTCAGCACTCAGTTATTGGGTCTCCCTCATGCCTTTTCATATACTTTC 5600  
 I L F L W V L . M P S Q H S V I G F S P H A F H I L S  
 S Y F C G S Y R C Q V S T Q L L G S P L M P F I Y F  
 Y L I F V G L I D A K S A L S Y W V L P S C L S Y T F

TTATCTACTGCCTTTGGGAGATAGTCTTATGTAGCCAGGCTGCTCTGATCTTGAATTGCTTGCTCAGCTTCTCA 5680  
 Y L L P F G R . S Y V A Q A V L D L G I C L P Q L L  
 L I Y C L L G D S L M . P R L S L I L E F A C L S F S  
 L S T A F W E I V L C S F G C P . S W N L L A S A S Q

GTCTCAAGTACTGGGATAATAGGCATGCATTGTCTGCCTGGCCTTTGCTGAACATGCCCTCTGTGGCCATTGGTAGGGCA 5760  
 S L K Y W D N R H A L S A W P L L N M P S V A I G R A  
 V S E S T G I I G M H C L P G L C . T C P L W P L V G H  
 S Q V L G . A C I V C L A F A E H A L C G H W . G

TGAGTCAAACTACTGCCCTCCCCACACACACACAAACGAAAGTGAGGCTCTCTAAGTGTTCATAGCAGCAGGGTAGT 5840  
 . V K Y C P P P Q H T H K R K . G S L S V P . H R V V  
 E S N T A L P S P T T H T N E S E A L . V F H S T G .  
 M S Q I L P S P T T H T Q T K V R L S K C S I A Q G S

GGTAGGCCTCTCGTAGTGATATTTCTTCTTTACTCTGCCATCTCTTCTTTGATTTCACACTGGGGACCTG 5920  
 V G L S L V H I S F F Y S A H L F F L . F P H W G F  
 W . A S R . C I F H S F T L P I S S F F D F H T G D L  
 G R P L A S A Y F I L L L C P S L L S L I S T L G T W

GCATAGTACTTTCTGTGAATTAAGAGAGAATTCCTTTTAAAGTGCTGCATTGCAGCGTCTCCTGGGACATTCTCCT 6000  
 G I V L S W . L R E N S L L S A C I A A S S W D I L P  
 A . Y F P G N . E R I P F . V P A L Q R P P G T L F S  
 H S T F L V I K R E F P F K C L H C S V L L G H S F

TGCTGACTACACCCACATCCTTCCATGTTTTTGTTCCTCATCTATGCCCTTCTAGGCTGTCCACATACATGG 6080  
 C . L H F T S F H V F C F P S L C P P S R L S H I H G  
 A D Y T P H P S M F F V S H H Y A P L L G C P T Y M  
 L L T P H I L P C F L F P I T M P P F . A V F H T W

ATGTCGTCTTGTGTTGGATGGCTCCAACAGTATCTATCCCTGGTCAGAAGTTCAGACTTTCTTGGAGGCTGGTAGGA 6160  
 C R H C F G W L Q Q Y L S L V R S S D F P S E A G R  
 D V I V L L D G S N S I Y P W S E V O T F L R L V G  
 M S S L F W M A P T V S I P G Q K F R L S F G G W . E

AGACTGTTTCATCGATCCGGAGCAGATACAGGTAAGAGAAGATATGTGGATAGGATTGGAGGGAAGAAGTAAACACTCC 6240  
 N T V H R S G A D T G K R K I C G . D W R E R S K N H S  
 R L F I D P E I O V R E R Y V D R I G G K E V N T P  
 D C S S I R S R Y R . E K D M W I G L E G K K . T L

TGGACCTTGGATGTAAGCAGCATGTCAGCCTCTTGTGACACCTGGGACATTGTCTTACAGAACTCATGCTCAA 6320  
 W T L G C K Q P C P A S . . H P G T L S S T E L M L K  
 G P L D V S S H V Q P L D D T L G H C L L Q N S C S  
 L D P W M . A A M S S L L M T P W D I V F Y R T H A O

GAACTGTGCAATTAACCTACAAAAAGTCACAAAAATTTATAATGTTGAAGTAAGTTTATGATTGTGTGGGGGCCAC 6400  
 N C A I N L P K S H K N F I M F E V S L . L C G G F  
 R T V Q L T Y Q K V T K I S . C L K . V Y D C V G G H  
 E L C N . L T K K S Q K F H N V . S K F M I V W G A T

ACTCAGAGCTTCCCTTTGCTGCTTGTAGTTGCTTGGGCAATGCATGCCATGAGCTGCAAGTTAGACACACCTGTTCACTT 6480  
 H S E L P F A A C S C L G N A C H E L Q V R H T C S L  
 T Q S F P L L L V V A W A M H A M S C K L D T P V H F  
 L R A S L C C L . L L G Q C M P . A A S . T H L F T

CCCTTCATCGTCTGCAGGTGGACACACCTGTTAGGGGTTCACTTCCCTTCATCCTTTGTGCTCCATCTTCTCTACG 6560  
 P L H R A A A G W T H L L G V H F P F I L C A P S S L R  
 P F I V L Q V G H T C . G F T S P S S F V L H L L Y  
 S P S S C C R L D T P V R G S L P L H P L C S I F S T

CTCTTCATACATCCCATGTGGGCACATGCTATTGTTCTCAGGTAGGACTGGTACAGTACGGGGAGAACCTGTGTCATG 6640  
 S S Y I P C G H M V Y C S Q V G L V Q Y G E N P V H  
 A L H T S H V G T W S I V L R . D W Y S T G R T L C M  
 L F I H F M W A H G L L F S G R T G T V R G E P C A .

AGTGGTCCCTGGGAGACTTCCGAACAAAGGAAGTTGTGAGAGCAGCAAGGAACCTAAGTCGGAGGGAAGGGCGAGAA 6720  
 E W S L G D F R T K E E V V R A A R N L S R R E G R E  
 S G P W E T S E Q R K K L . E Q Q G T . V G G K G E K  
 V P K G R L P N K G R S C E S S K E P K S E G R A R

ACGAGAACCCGCAAGCATGCTGGCATGGTGAGACATTGTAAAGGGGTGCTGTGAGGGAGGAGGAAGGATCAGCAG 6800  
 T P T A Q A I M V A W . D I V K G S C E G G G R I S R  
 R E P P K R S W W H G E T L . R G R V R E E E G S A  
 N E N R P S D H G G M V R H C K G V V . G R R K D Q Q

FIGURE 15e

FIGURE 15f

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1 2 3 4

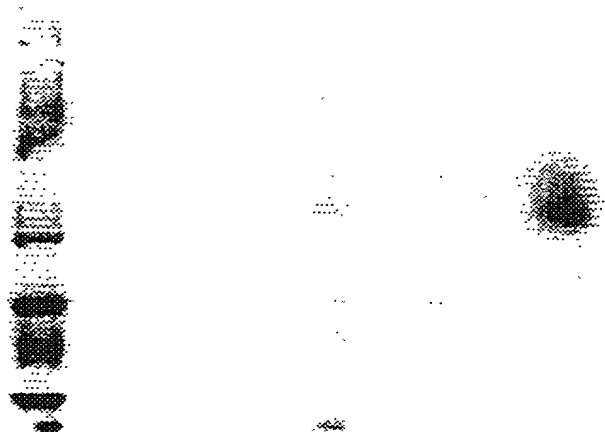


FIGURE 16

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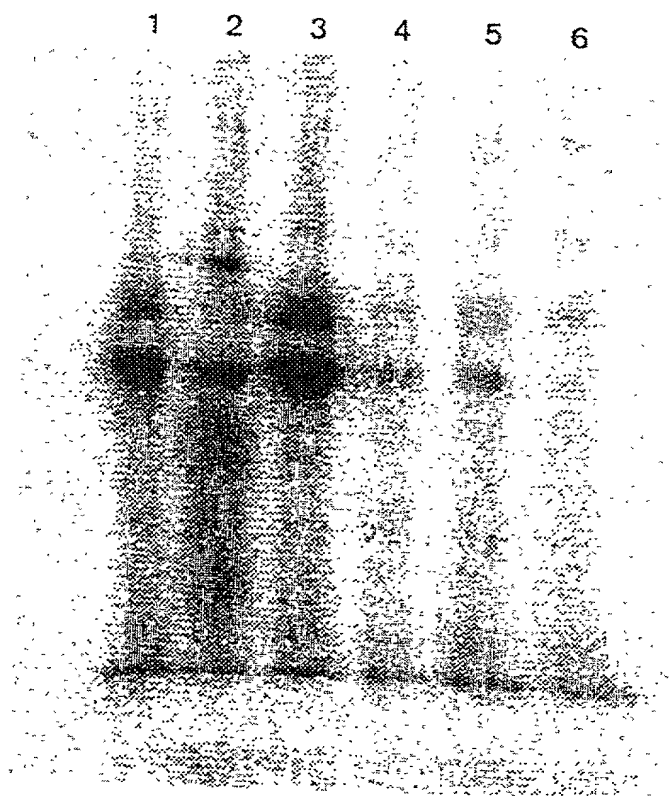


FIGURE 17

**COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY**  
(Includes Reference to Provisional and PCT International Applications)

Attorney's Docket No.

003300-685

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**An integrin heterodimer and a subunit thereof**

the specification of which (check only one item below):

☐ is attached hereto.

☒ was filed as United States application

Number

on **2 October 2000**

and was amended

on \_\_\_\_\_ (if applicable).

☐ was filed as PCT international application

Number

on

and was amended

on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

**PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:**

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
Sweden	9801164-6	2 April 1998	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Sweden	9900319-6	28 January 1999	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

\_\_\_\_\_  
(Application Number)

\_\_\_\_\_  
(Filing Date)

\_\_\_\_\_  
(Application Number)

\_\_\_\_\_  
(Filing Date)

<b>COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED)</b> (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No. 003300-685
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I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to the patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS		STATUS (check one)		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED
PCT APPLICATIONS DESIGNATING THE U.S.				
PCT APPLICATION NO.	PCT FILING DATE	U.S. APPLICATION NUMBERS ASSIGNED (if any)		
<b>PCT/SE99/00544</b>	<b>31 March 1999</b>			

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

William L. Mathis                      17,337 Robert S. Swecker                    19,885 Platon N. Mandros                    22,124 Benton S. Duffett, Jr.                22,030 Norman H. Stepno                    22,716 Ronald L. Grudziecki                24,970 Frederick G. Michaud, Jr.          26,003 Alan E. Kopecki                      25,813 Regis E. Slutter                      26,999 Samuel C. Miller, III                27,360 Robert G. Mukai                    28,531 George A. Hovanec, Jr.            28,223 James A. LaBarre                   28,632 E. Joseph Gess                      28,510	R. Danny Huntington               27,903 Eric H. Weisblatt                    30,505 James W. Peterson                  26,057 Teresa Stanek Rea                  30,427 Robert E. Krebs                    25,885 William C. Rowland                30,888 T. Gene Dillahunt                  25,423 Patrick C. Keane                    32,858 Bruce J. Boggs, Jr.                32,344 William H. Benz                    25,952 Peter K. Skiff                      31,917 Richard J. McGrath                29,195 Matthew L. Schneider            32,814 Michael G. Savage                  32,596	Gerald F. Swiss                      30,113 Michael J. Ure                      33,089 Charles F. Wieland III            33,096 Bruce T. Wieder                    33,815 Todd R. Walters                    34,040 Ronni S. Jillions                  31,979 Harold R. Brown III                36,341 Allen R. Baum                      36,086 Steven M. du Bois                  35,023 Brian P. O'Shaughnessy          32,747
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**21839**

and: None

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-28		COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED) (Includes Reference to Provisional and PCT International Applications)		ATTORNEY'S DOCKET NO. 003300-685	
FULL NAME OF SOLE OR FIRST INVENTOR <b>Evy Lundgren-Akerlund</b>		SIGNATURE <i>Evy Lundgren-Akerlund</i>		DATE 2010-10-09	
RESIDENCE <b>Bjärred, Sweden</b> SEX				CITIZENSHIP Sweden	
POST OFFICE ADDRESS <b>Trollsjövägen 165, 237 33 Bjärred, Sweden</b>					
FULL NAME OF SECOND JOINT INVENTOR, IF ANY		SIGNATURE		DATE	
RESIDENCE				CITIZENSHIP	
POST OFFICE ADDRESS					
FULL NAME OF THIRD JOINT INVENTOR, IF ANY		SIGNATURE		DATE	
RESIDENCE				CITIZENSHIP	
POST OFFICE ADDRESS					
FULL NAME OF FOURTH JOINT INVENTOR, IF ANY		SIGNATURE		DATE	
RESIDENCE				CITIZENSHIP	
POST OFFICE ADDRESS					
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY		SIGNATURE		DATE	
RESIDENCE				CITIZENSHIP	
POST OFFICE ADDRESS					
FULL NAME OF SIXTH JOINT INVENTOR, IF ANY		SIGNATURE		DATE	
RESIDENCE				CITIZENSHIP	
POST OFFICE ADDRESS					
FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY		SIGNATURE		DATE	
RESIDENCE				CITIZENSHIP	
POST OFFICE ADDRESS					
FULL NAME OF EIGHTH JOINT INVENTOR, IF ANY		SIGNATURE		DATE	
RESIDENCE				CITIZENSHIP	
POST OFFICE ADDRESS					
FULL NAME OF NINTH JOINT INVENTOR, IF ANY		SIGNATURE		DATE	
RESIDENCE				CITIZENSHIP	
POST OFFICE ADDRESS					